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PINEALOMA

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The term "pinealoma" is used to designate any tumor occurring in the pineal region and any neoplasm arising elsewhere in the brain provided the origin can be traced histologically to the pineal body. The term also carries with it certain restrictions and is to be withheld from a tumor in the pineal region if the tumor is histologically not of pineal derivation.

For some time the true character of new growths of this type was not understood. This was primarily due to the great variability in the cellular structure and to a certain inconstancy in the location of the tumors. These observations made it difficult to identify the tumors as members of the same morphologic group, resulted in a multitude of interpretations of their histologic character and gave rise to a correspondingly large assortment of names. This is shown in the survey made by Haldeman [1] of practically all of the pineal tumors reported in the literature since 1800. He found them described as sarcoma, fibrosarcoma, adenoma, chorionepithelioma, psammoma, adenocarcinoma, teratoma, glioma, angiosarcoma, mixed tumor and ependymal glioma. In each instance the name was adopted because of some prominent feature of the given tumor, and, unfortunately, in the majority of instances its adoption was based on incomplete studies.

But as the important observations of Marburg [2] and Krabbe [3] on the histogenesis of the pineal body became available, the way was open for the recognition of the classic structural pattern of the typical pinealoma. Subsequent studies of Globus and Silbert [4] provided for the establishment of a common link joining the many apparently structurally divergent tumors found in the immediate vicinity of the pineal

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1. Haldeman, K. O.: Arch. Neurol. & Psychiat. **18**:724, 1927.
2. Marburg, O.: Arb. a. d. neurol. Inst. a. d. Wien. Univ. **17**:217, 1908.
3. Krabbe, K. H.: Anat. Hefte **54**:191, 1916.
4. Globus, J. H., and Silbert, S.: Arch. Neurol. & Psychiat. **25**:937, 1931.

body or less commonly in territories somewhat distal to it. Globus and Silbert were able to show that the structural patterns which typify certain critical histogenetic stages in the development of the pineal body can be matched with the cellular patterns of some tumors growing in or about the quadrigeminate plate (pineal region) and may be used as leads for the identification of such tumors as members of the same family, now designated as pinealoma.

The material to be presented here is offered to demonstrate the usefulness of these observations and to show how tumors of pineal origin which present divergent histologic organization can be identified as neoplasms which have a common stem of origin. Since the morphologic unity of the varied histologic features of the pineal tumors is based on the understanding of the progressive stages in the prenatal and postnatal development of the pineal body, this aspect of the study will be discussed first.

HISTOGENETIC PHASES IN THE DEVELOPMENT OF THE HUMAN PINEAL BODY

The primordium of the pineal body in the human embryo is first noted at the beginning of the second month. It is recognized as a rather shallow evagination, called the pineal diverticulum (fig. 1 *A*), of the roof of the second brain vesicle (diencephalon) at a point where it joins the third secondary vesicle (mesencephalon). Directly in front of this evagination there is assembled a small group of cells, constituting the anterior pineal anlage (primordium) (fig. 1 *B*). The cells lining this diverticulum are much like those in the roof of the developing brain. The cells constituting the anterior pineal anlage resemble the cells within the walls of the growing brain and are characterized by a large oval or round nucleus enveloped by scanty cytoplasm. They are densely packed except at the center of the anlage, where they form a syncytium-like protoplasmic mass.

From the end of the second and through the third embryonal month little change takes place except that the diverticulum is deeper (fig. 2 *A*) and shows several secondary indentations at its base. It is this diverticulum, including both its anterior and posterior walls, with the addition of a small cellular mass caudal to it, which forms the so-called posterior pineal primordium or anlage (fig. 2 *B*). At this time the anterior pineal anlage, which has increased in size, maintains its proximity to the pineal diverticulum and is separated from the latter only by a narrow space occupied by loose connective tissue and blood vessels.

During the fourth month of fetal life there is a notable increase in the size of both pineal primordia (anlagen). The cavity in the diverticulum narrows at its opening but widens in its depth.

In the course of the fifth fetal month, fusion of the anterior and posterior pineal primordia takes place (fig. 2 *C*). Now the pineal body begins to assume the conical outline which characterizes it in adult life, and its long axis acquires a direction parallel with the underlying aqueduct of Sylvius. The diverticulum is quite narrow. The cell type

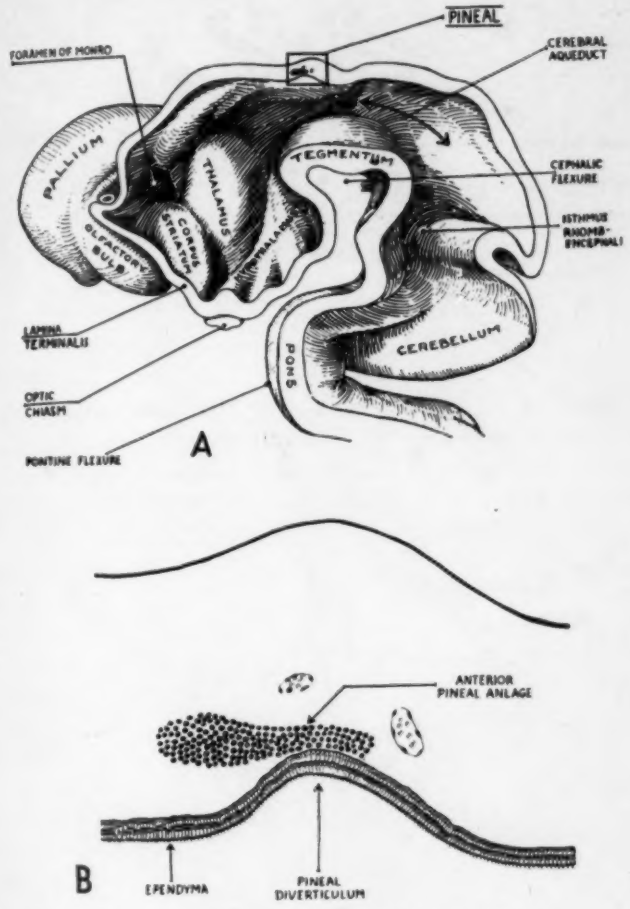

Fig. 1.—*A*, location of pineal premordium in a fetus of 2½ months. Drawing after His. *B*, relation of anterior pineal anlage to the pineal diverticulum in a fetus of 2½ months. Drawing after Krabbe.[3]

and arrangement display little change except that the cells lining the entrance of the diverticulum show elongating nuclei and fine cytoplasmic striations.

At the beginning of the sixth prenatal month a more definite change in the organization of the pineal body becomes apparent; it now seems

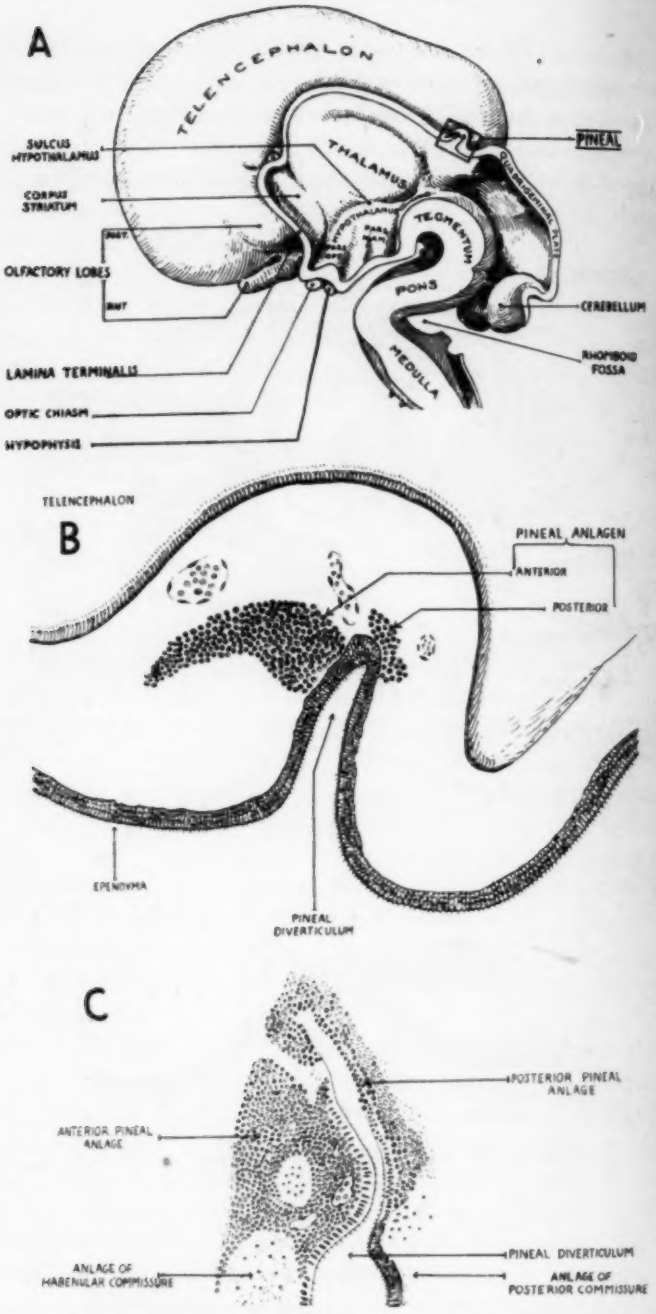

Fig. 2.—*A*, pineal anlage in a fetal brain at 3 months. Drawing after Corning (Lehrbuch der Entwicklungsgeschichte, Munich, J. F. Bergmann, 1921). *B*, relation of the pineal anlage (anterior and posterior) to the pineal diverticulum in a fetus of 3 months. *C*, pineal primordia (anlage) and pineal diverticulum in a fetus of 4½ months.

to acquire a resemblance to a glandular structure (fig. 3 *A*). In the region roughly identified as corresponding to the anterior pineal primordium there are visible several large tubule-like structures.

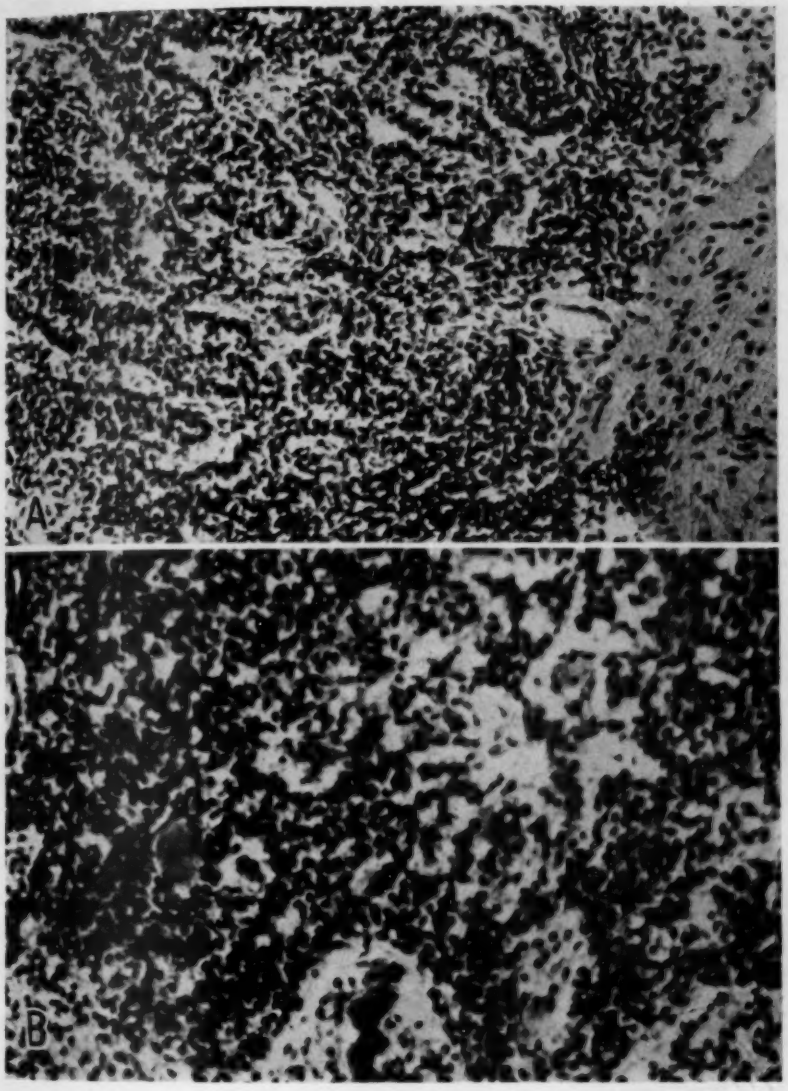

Fig. 3.—*A*, section of the pineal body in a 5½ month fetus (photomicrograph). The pineal body appears as a fairly large bud consisting of cuboid and columnar cells lining a glandlike structure. *B*, section of the pineal body in a 6½ month fetus (photomicrograph). A definite structural pattern is now apparent, produced by dense cords of deeply stained cells encircling clearer zones containing fewer but larger and faintly stained cells, enclosing vascular channels.

Directly caudad to these there is a large number of smaller alveoli-like cellular collections. The former are lined by columnar cells, not unlike those lining the diverticulum, while the "alveoli" are mapped out by rings of low cuboid cells. The diverticulum is sealed off in the middle of its course by a cellular mass, and is thus converted in its deeper part into a closed space, the pineal cavum, and into the pineal recess.

The next definite structural alteration is noted in the pineal body of a fetus at about 6½ months. Here masses which assume in places the character of solid cords of densely stained small cells encircle lighter zones containing relatively few cells, faintly stained (fig. 3 *B*). The lighter zones are traversed by vascular channels, which usher in the stage of vascularization. This is the period in which the mosaic structure which will characterize the late prenatal and early postnatal developmental stages first becomes apparent.

In the fetus at 7½ months the so-called lighter areas show an increase in the number and size of their cells (fig. 4 *A*) and the vessels, which were prominent in the previous stage, are somewhat less conspicuous and are displaced to the periphery.

In the 8½ month fetal pineal body another stage of differentiation becomes apparent. The pattern that is to characterize the structure of this organ during late prenatal and early postnatal life comes into full view. Streams of small round cells with deeply staining nuclei and scanty cytoplasm break up the pineal territory into irregular polygons, each enclosing cell masses of lesser staining intensity. The cells within the latter are easily distinguished from the small dark cells in the encircling streams by their large size and their poorer staining qualities. The large cells already in this stage of development show features simulating those of the mature parenchymal cells of the adult pineal body.

This pattern of the pineal body comes into greater display during the last days of fetal life and first few days of postnatal life. The characteristic pattern of clear areas surrounded by narrow bands of deeply staining cellular elements is readily recognized under low magnification (fig. 4 *B*) and is maintained to about the end of the third week in the postnatal development of the pineal body (fig. 5 *A*). While there is no obvious diminution in the number of the small cells, the large cells increase in size and probably also in number; the blood vessels are found mainly in the clear parenchymal areas, a feature which suggests a probable glandular function of the organ at this stage of its development.

With the completion of the first month of postnatal life, the pineal body enters a new series of developmental stages. The mosaic character of its structure begins to fade. The small cells begin to break up the continuity of their streamlike formation and diminish in number,

as seen in the pineal body in about the ninth postnatal week (fig. 5 *B*). This is still more pronounced at the end of four months of postnatal life (fig. 6 *A*).

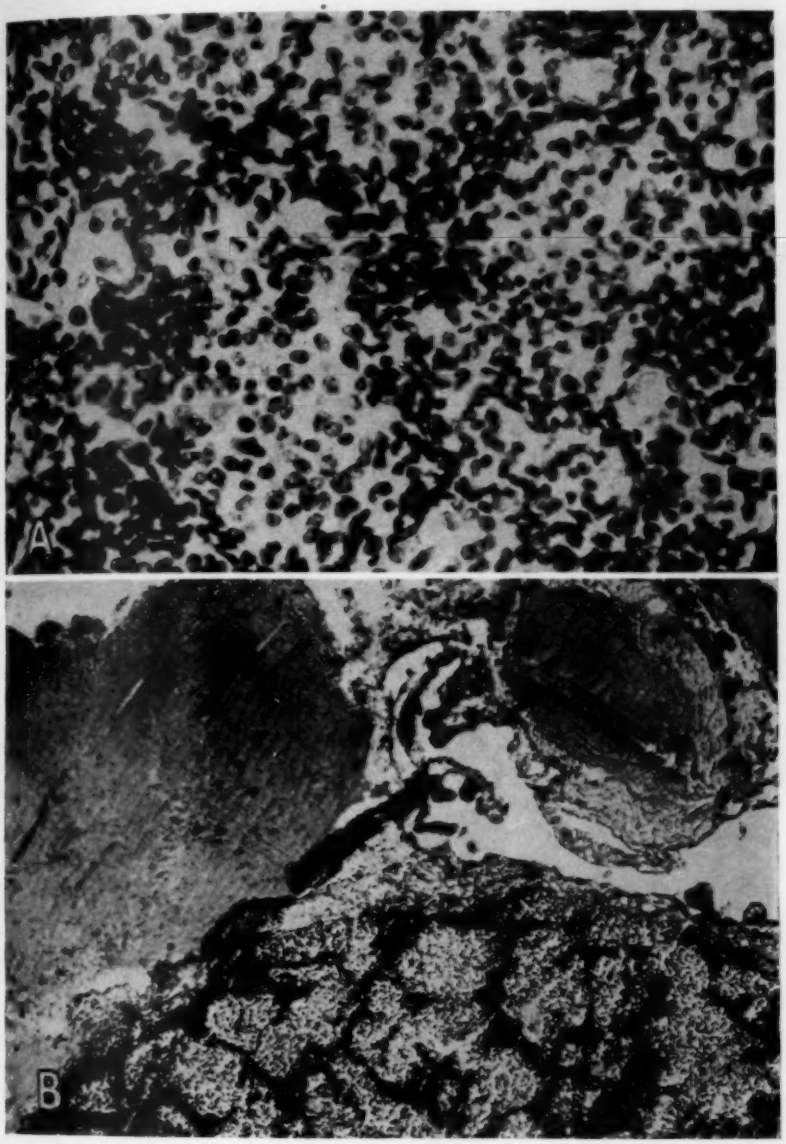

Fig. 4.—*A*, section of the pineal body in a 7½ month fetus (photomicrograph). The clearer zones show an increase in the number and size of their constituent cells. *B*, sagittal section of the pineal body in an infant 1 day old (photomicrograph). The differentiation of the two zones is more distinct, imparting a mosaic pattern to the pineal body. Note the two large vessels at the periphery.

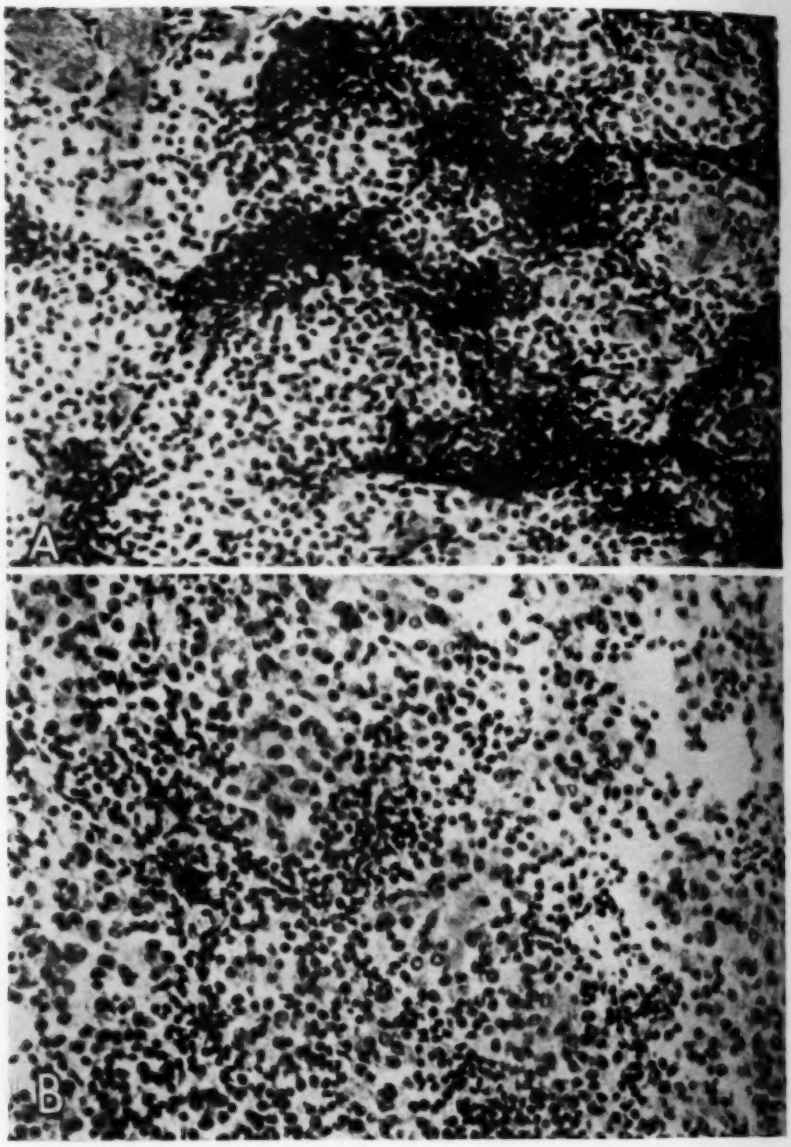

Fig. 5.—*A*, section of the pineal body in an infant 19 days old (photomicrograph). The mosaic pattern reaches the height of its development. The large cells in the clearer zones are increasing in size. *B*, section of the pineal body in an infant 9 weeks old (photomicrograph). The small dark cells have decreased considerably in number, resulting in a distinct loss of the mosaic pattern present in the earlier stages.

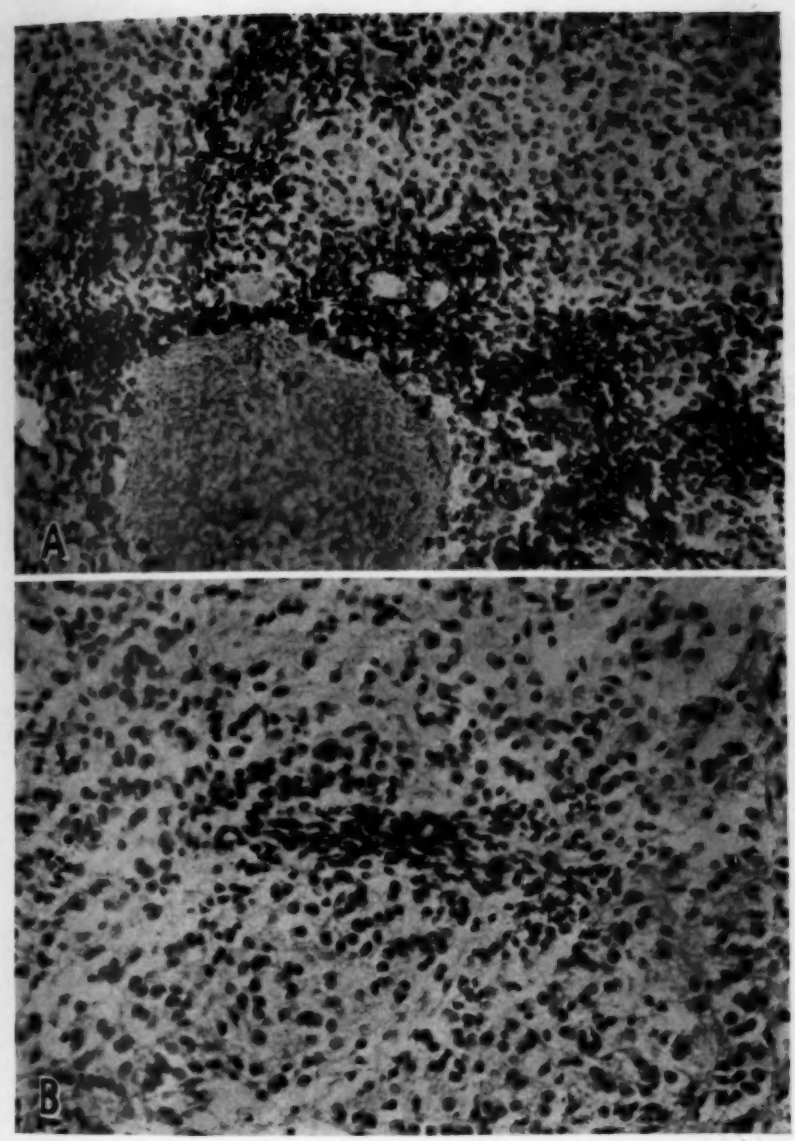

Fig. 6.—*A*, section of the pineal body in an infant 4 months old (photomicrograph). A more pronounced decrease in the number of the small dark cells leads to complete loss of the mosaic pattern. *B*, section of the pineal body in an infant 5 months old (photomicrograph). The remaining few small dark cells have now become elongated and gradually assume the form and staining reaction of fibroblasts.

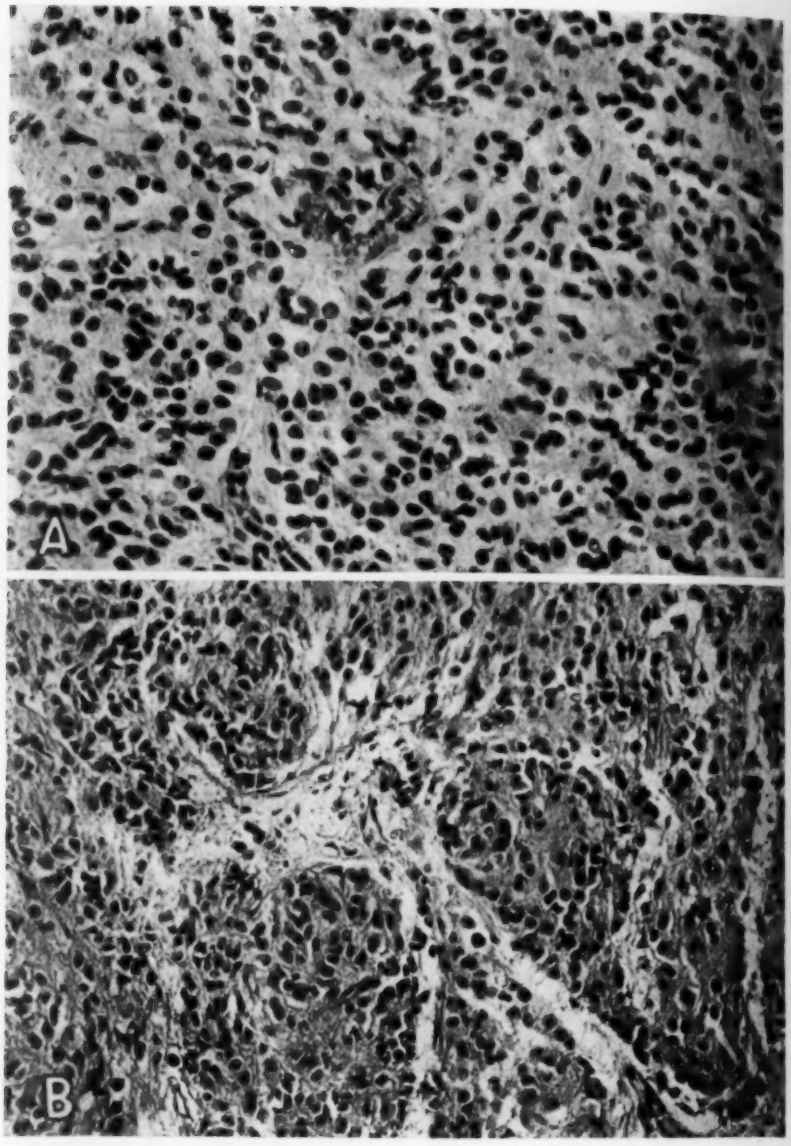

Fig. 7.—*A*, section of the pineal body in a child 18 months old (photomicrograph). At this stage the pineal body gradually assumes the adult form; the cell arrangement assumes an alveolar pattern, with the fibroblastic elements contributing to the formation of connective tissue trabeculae. *B*, section of the pineal body in a child 5½ years old (photomicrograph). The structure of the pineal body is approaching full maturity. The parenchymal cells are grouped in fairly solid masses and give rise to lobules, surrounded by an abundance of supporting connective tissue.

After the fifth postnatal month only small islands of the dark cells are in evidence. They assume a new character, becoming elongated, and approach the form of fibroblasts, which are later apparently converted to or replaced by connective tissue, as demonstrated by staining

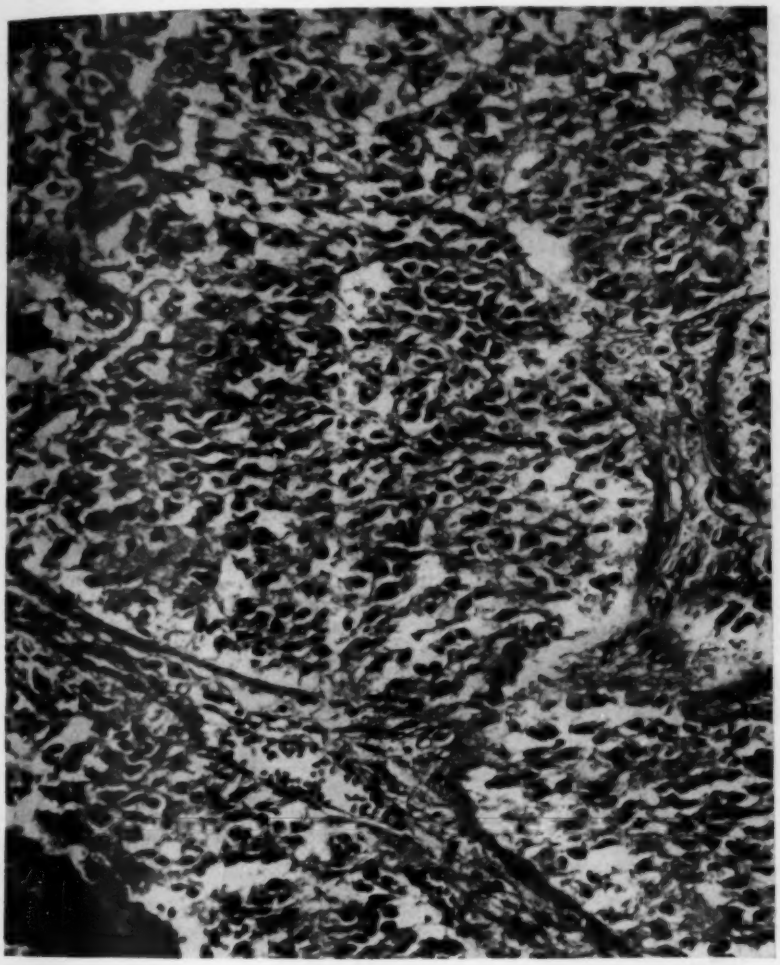

Fig. 8.—Section of a fully matured pineal body in an adult 42 years old (photomicrograph).

reactions (fig. 6*B*). This change continues, so that at the end of nine months little is left of the old mosaic structure. The dark cells are now found in exceedingly small groups, with here and there a few isolated cells suggesting the previous larger accumulations. In somewhat later stages the pattern so characteristic of the early days of postnatal life

is no longer recognizable. Only an occasional accumulation of small cells is found in isolated areas.

Another transition in the organization of the pineal body appears to take place at about the end of the eighteenth month of postnatal development. The parenchymal cells now seem to acquire an alveolar arrangement; they are separated from one another by connective tissue trabeculae (fig. 7 *A*). At a little later period there are noted groups of basophilic cells and of small mast cells, alongside which there is an occasional large eosinophilic cell.

From this period on, the structural changes are slight and occur over long periods. At five and a half years the structure of the pineal body acquires a lobulated appearance, with solid nests of cells being surrounded by strands of connective tissue (fig. 7 *B*). From then on until full maturity further changes are gradual and are mainly of a regressive character. They are characterized by increase in interlobular fibrosis, with the formation of cysts, and deposition of calcium (fig. 8).

ANATOMIC FEATURES OF PINEALOMA

With the foregoing observations on the several histogenetic phases of the developing pineal body in mind, one can more readily understand the diversity in the structure of pineal tumors. Little difficulty will be experienced in recognizing the typical example of pinealoma with its mosaic cellular arrangement, in which nests of faintly stained parenchymal cells encircled by streams of dark-stained smaller cells mirror the structure of the pineal body in its first month of postnatal development. Nor will there be great difficulty in following the deviations from this typical structural pattern revealed by less typical examples of pinealoma which recapitulate structurally either earlier or later stages in the development of the pineal body. In all, 10 cases are to be described. Stress will be put mainly on the histologic character of the tumor, and attention will be drawn to the features which link the growth to one or another histogenetic stage in the normally developing pineal body.

CASE 1.—*A typical pinealoma in a boy of 13 with a history of frequently recurrent headache since the age of 10, accompanied by the development of clinical signs of an expanding intracranial lesion in and about the region of the quadrigeminal plate.*

Gross Anatomy.—The surface of the brain shows evidence of a marked increase in intracranial pressure. At the base of the brain the tuber cinereum is found markedly distended and thinned out. Separation of the cerebral hemispheres brings into view a large mass, measuring about 2.5 cm. in its long diameter, and protruding above the dorsal surface of the cerebellum. It is found infiltrating the quadri-

geminal plate anteriorly (fig. 9*A*) and the anterior border of the cerebellum posteriorly; laterally it extends into the cerebellar hemispheres and the right side of the midbrain. The pineal body cannot be found.

Microscopic Anatomy.—This tumor was selected as the first to be described because of its histologic structure, which is typical of the classic form of pinealoma.

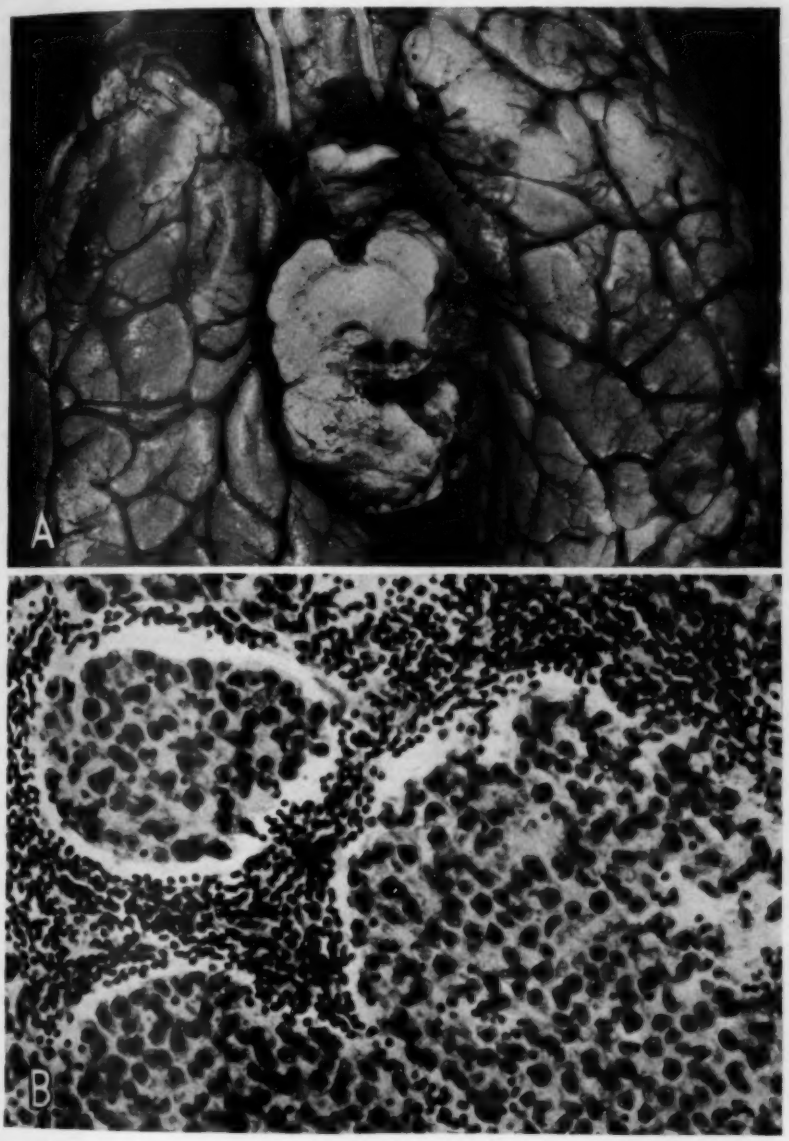

Fig. 9.—*A*, tumor situated in the quadrigeminal plate directly above the aqueduct of Sylvius (case 1). *B*, section of the tumor showing the typical mosaic pattern (photomicrograph).

It is characterized by the striking cell organization, with streams of small, deeply stained cells encircling nests of larger, lightly staining cells. The latter, because of their size, the vesicular character of their nuclei and the larger rims of clear cytoplasm (fig. 9 *B*), reflect a distinct cell type bearing a strong resemblance to the parenchymal cells of the mature pineal body. The arrangement of these two cell forms, small and large, respectively, dark and light, give the tumor a mosaic cell pattern such as is revealed in a survey of the various histologic pictures presented by the pineal body in its postnatal evolution. It will be noted, then, that this pattern is a close imitation of patterns seen in the pineal bodies of infants from 3 to 8 weeks of age (figs. 4 *B* and 5 *A*). Moreover, it invites the assumption that this tumor in its histologic makeup recapitulates one or more chapters in the histogenesis of the pineal body, and may suggest the possibility that tumors of this type take their origin from embryonal rests.

While the cell grouping thus far described forms the dominant histologic picture noted in the larger part of the tumor, there are also fields which show deviations from the typical cellular organization. In such areas, while the large cells seem to retain their outline and number, the small cells show a marked reduction in number, being apparently replaced by fibroblasts and collagenous fibers. It is not improbable that the collagenous fibers develop at the expense of the small round cells, as young fibroblasts can often be seen to develop among the gradually disappearing small dark cells. It is further demonstrated in silver preparations that wherever the small cells are in large numbers there are few fibers but that as these cells begin to disappear fine fibers come into view in large numbers.

Giant cells are not uncommon in some parts of the tumor. They are usually more often found in the vicinity of calcified plaques. They commonly have the appearance of foreign body giant cells and occasionally contain enclosures. No ganglion cells are noted in the tumor tissue, although it is not improbable that such cells may occur when the tumor invades the brain stem.

CASE 2.—*A pinealoma, fairly typical in structure, in a boy of 17, who gave a history of polydipsia and polyuria of one year's duration, followed by signs of increased intracranial tension and evidence of an expanding lesion in and about the quadrigeminal plate.*

Gross Anatomy.—The brain is voluminous and under increased pressure. The tuber cinereum is very thin. When the occipital lobes are raised, a gelatinous soft mass is found covering the roof of the aqueduct and part of the fourth ventricle. The pineal body cannot be found. The ventricles in front of the aqueduct are much distended. The quadrigeminal plate is replaced by a large mass of soft, friable and granular tissue (fig. 10 *A*). This mass measures about 2 by 2 by 3 cm.; embedded in the depths of the mass is a small calcareous body, which is probably a remnant of the pineal body. The midbrain is markedly distorted, particularly in the region of the tectum and part of the tegmentum; the nucleus ruber is small, and the substantia nigra is poor in pigment. The aqueduct is large and filled with a soft matter, while the fourth ventricle is of normal size but is filled with similar material.

Microscopic Anatomy.—The cellular organization of this tumor is almost identical with that in case 1 and needs no detailed description. Here again the characteristic mosaic is present. The small cells form streams which envelop nests of clear parenchymatous cells (fig. 10 *B*). The only difference is the smaller number of the dark, deeply stained round cells and the larger size of the clearer

parenchymal cells. The transition of the small round cells into fibroblasts is also present, but to a lesser degree. Mitotic figures are frequently seen in the large parenchymal cells. Giant cells of the foreign body variety are also present, most frequently in the neighborhood of deposits of calcium.

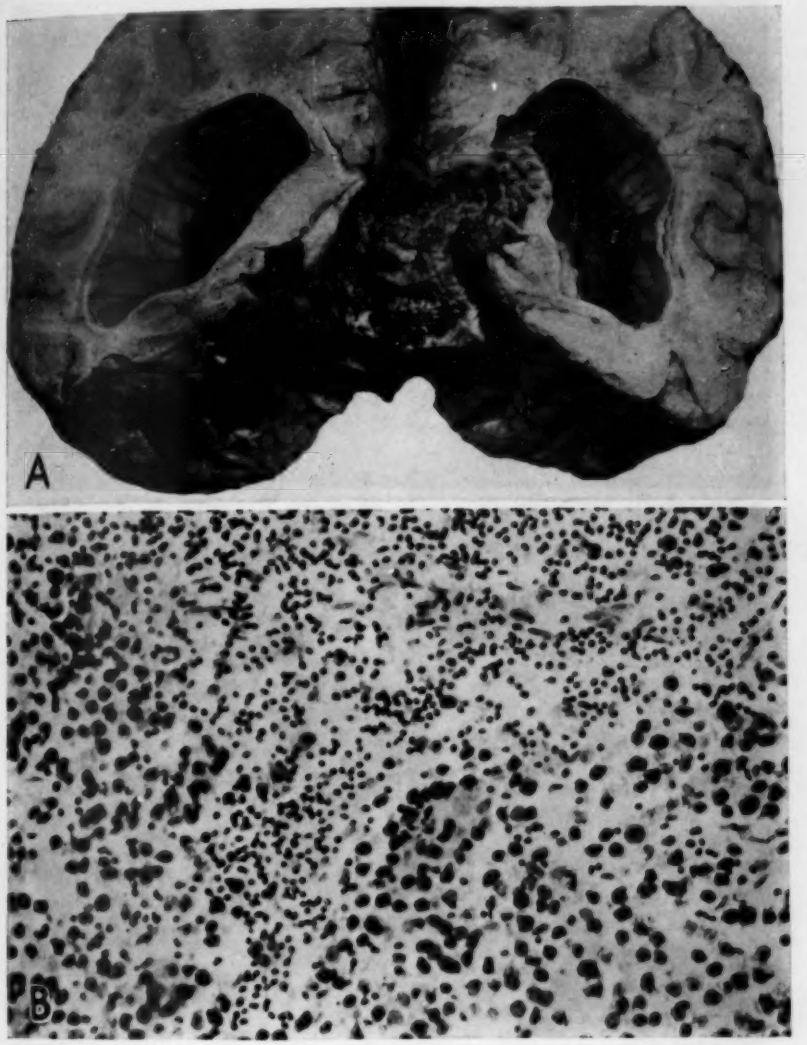

Fig. 10.—*A*, gross appearance of pinealoma in case 2 (photomicrograph). *B*, section of this tumor illustrating its histologic character. The two cell types are less regular in arrangement than those in case 1.

CASE 3.—*A pineal tumor displaying structural residuals of the typical pinealoma in a boy of 13, who gave a history of headache of two years' duration, followed at a later date by development of signs pointing to involvement of the quadrigeminal plate and manifestations of increased intracranial tension affecting the hypothalamus.*

Gross Anatomy.—The brain shows evidence of increased intracranial tension. The right cerebral hemisphere is large and bulges in the right parieto-occipital region. The ventricles are greatly dilated, the right ventricular system somewhat

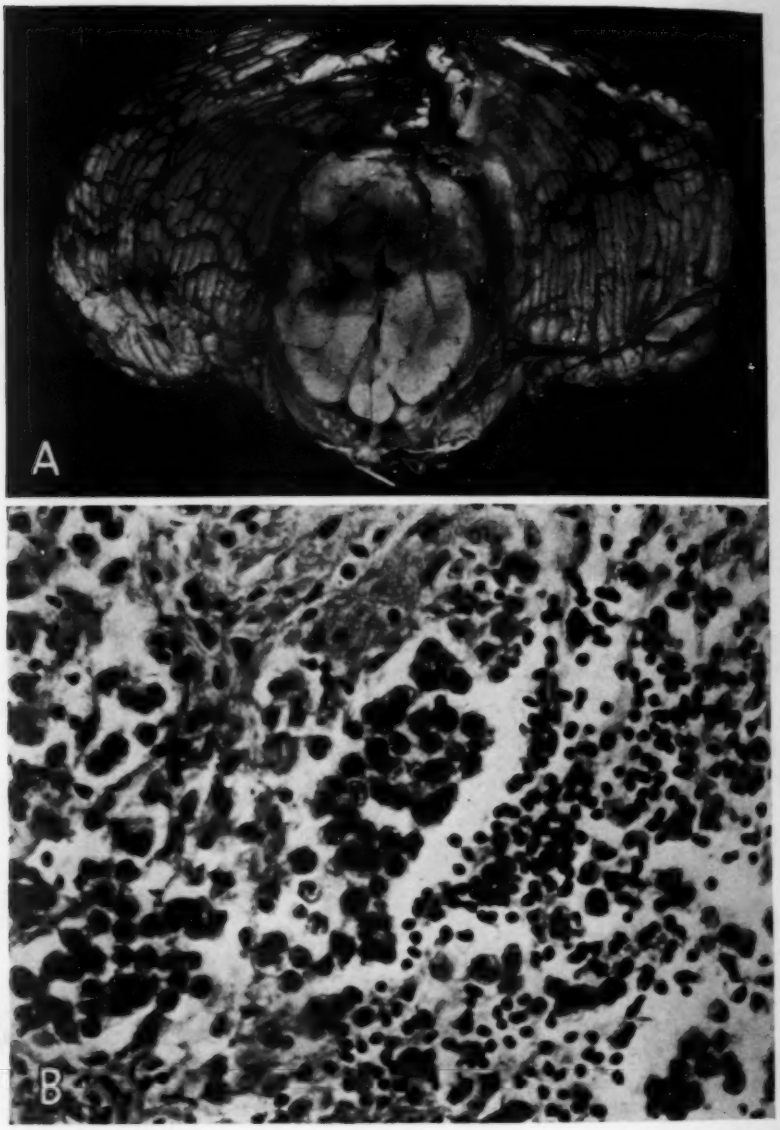

Fig. 11.—*A*, photograph showing location and invasive character of the pinealoma in case 3. *B*, section of the pinealoma, showing the two main cell forms without the characteristic mosaic structure (photomicrograph).

more than the left; the third ventricle is likewise dilated. The pineal body is large, calcified and embedded in a tumor which rests on the quadrigeminal plate and occupies the region of the anterior notch of the cerebellum (fig. 11 *A*). The tumor appears to invade the pulvinar on the left side. It measures about 2.5 cm. in its longest diameter.

Microscopic Anatomy.—The histologic picture shows a moderate departure from that noted in cases 1 and 2. On the whole, it displays a histologic structure indicative of an older, more differentiated phase in the development of the pineal body. Here the small round cells have almost completely disappeared (fig. 11 *B*), only a few of the cells being found at the periphery of some nests of parenchymatous cells. Their place, for the most part, is taken by robust bundles of collagenous fibers. In some areas groups of small cells embedded in fibrous stroma dominate the field, while in other areas connective tissue bundles with a few giant cells make the recognition of the true character of the tumor almost impossible. However, a thorough search through the material discloses areas in which the more characteristic pattern is readily recognized.

CASE 4.—*Pinealoma in the character of a pineal rest in the region of a partially obliterated aqueduct of Sylvius in a boy aged 16 years, who gave a history of recent onset of headaches, followed by other symptoms of increased intracranial tension and signs of involvement of the quadrigeminal region (including Argyll Robertson pupils). A ventriculogram revealed dilated third and lateral ventricles and a calcified shadow in the neighborhood of the posterior part of the third ventricle and the roof of the aqueduct.*

Gross Anatomy.—The gyri of the brain show marked flattening. There is considerable disorganization of tissue in the region of the dorsal surface of the midbrain and pons, the site of the operative intervention.

On sectioning, the following alterations are noted: (1) marked internal hydrocephalus affecting the lateral and the third ventricle, which show a certain amount of asymmetry, probably due to improper suspension in the fixing fluid; (2) marked disorganization of structures about the posterior part of the third ventricle and the posterior part of the left lateral ventricle; (3) some discoloration of the ependymal lining and marked swelling and disorganization of the choroid plexus; (4) disorganization of the entire left occipital lobe, which is traversed by channels of discolored and hardened areas that represent zones of extravasation which have undergone secondary hardening after fixation in solution of formaldehyde U. S. P.; (5) disturbance of the aqueduct of Sylvius, which is somewhat dislodged and at one point near its anterior end partly disorganized and partially broken into (the probability of atresia of the aqueduct of Sylvius is being considered as an explanation for the presence of the internal hydrocephalus).

A small piece of tissue, measuring 2 by 1 by 1 cm. and situated at the upper part of the posterior end of the third ventricle at the habenular commissure, was removed and sectioned. The rostral part of the midbrain was also sectioned in preparation for a microscopic study of the aqueduct of Sylvius.

Microscopic Anatomy.—Sections of the midbrain in the region of the aqueduct of Sylvius reveal tumor tissue with severe hemorrhages. The aqueduct is found to be partially obliterated by a small mass of tumor tissue (fig. 12 *A*) and in its remaining part is filled with desquamated ependyma, blood cells and macrophages. The tumor mass is quite cellular and displays a structural design much like that seen in the typical pinealoma or in the normal pineal body during its early postnatal development. The two types of cells are readily distinguished (fig. 12 *B*), one having a large pale-staining vesicular nucleus, the other, a smaller cell, con-

taining a small dark-staining nucleus. The former maintain a nestlike arrangement and are surrounded by somewhat irregular streams of the latter. The case strongly suggests the probability that an embryonal rest was responsible for this growth.

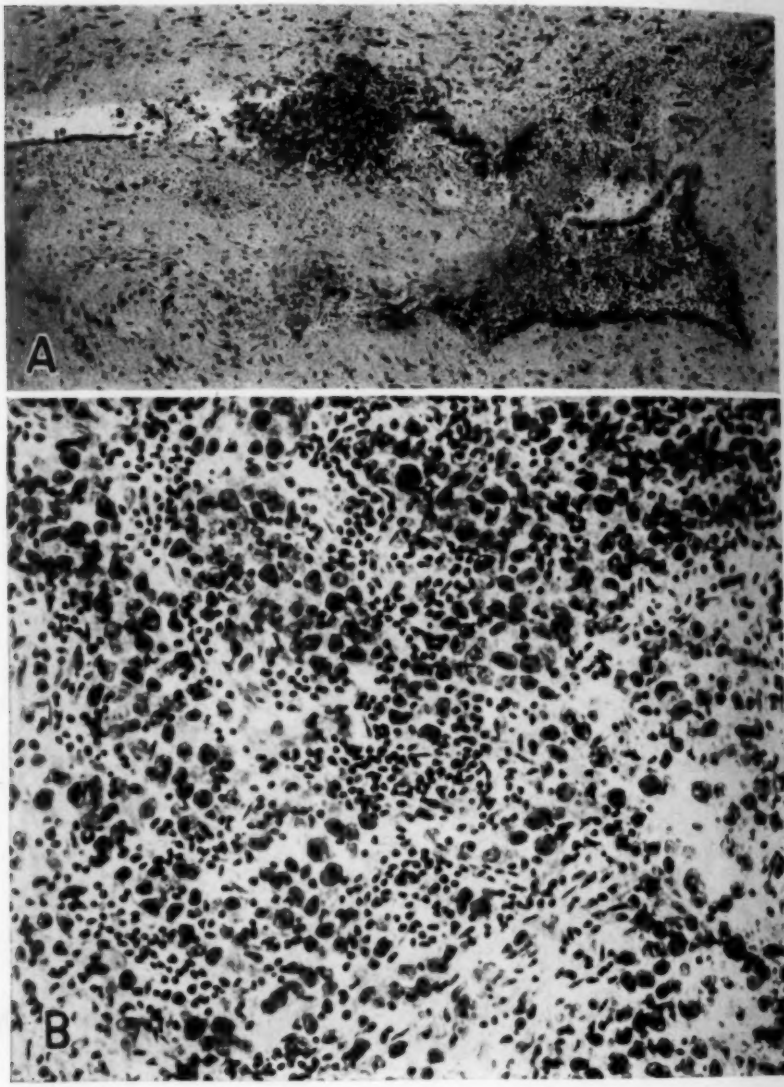

Fig. 12.—*A*, a small tumor mass in the proximity of an occluded and deformed aqueduct of Sylvius (case 4) (photomicrograph). *B*, higher magnification of the tumor mass shown, displaying the characteristic structure of a pinealoma (photomicrograph).

CASE 5.—*Pineal tumor displaying a wider departure from the structural design of a typical pinealoma but still presenting definite remnants of characteristic cell forms and cell arrangement alongside of other histologic formations recalling older*

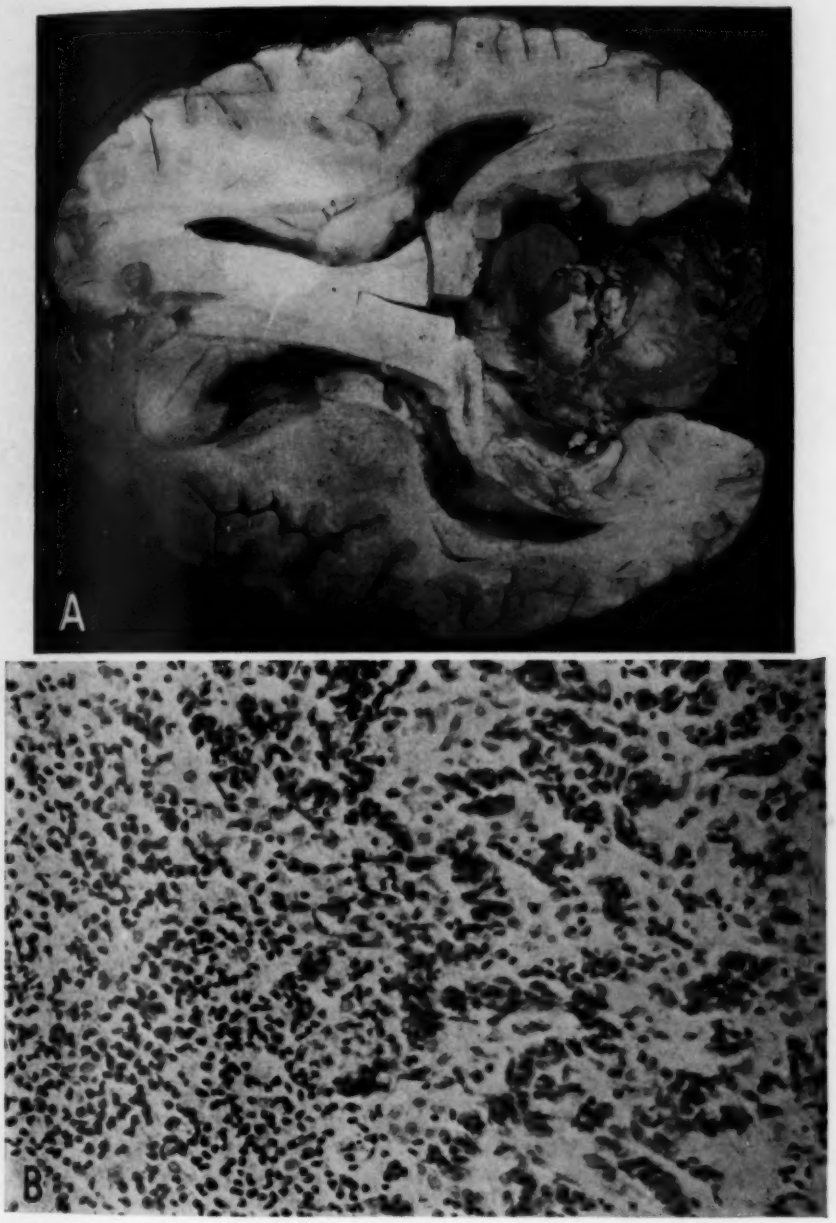

Fig. 13.—*A*, location of tumor in case 5. *B*, section of this tumor, showing the two cell forms; the small cells form a wide stream, bordered by zones of larger cells (photomicrograph).

stages of pineal differentiation. The patient was a man aged 30, who gave a history of a relatively recent (nine weeks) onset of signs of increasing intracranial tension, followed by generalized weakness, tremors, convulsive seizures, diplopia and incontinence. He presented signs of cerebellar, midbrain and pyramidal tract disturbance.

Gross Anatomy.—The brain is voluminous and under markedly increased tension. On the dorsal surface of the cerebellum in the region of the vermis there is a large mass (fig. 13 *A*) which extends laterally to both cerebellar hemispheres and forward as far as the quadrigeminal plate. It measures about 7 by 6 by 8 cm. The mass is encapsulated and separates easily from adjacent cerebellar tissue. It is irregular in outline, with several small nodules projecting from its dorsal surface. It is separated from the fourth ventricle by a thinned-out vermis, which is not invaded by the tumor mass but is depressed and flattened. The lateral ventricles display bilateral symmetric dilatation. The pineal body is found in its normal location and is of normal outline and size.

Microscopic Anatomy.—The structure of the tumor shows a still wider departure from the structural pattern of the typical pinealoma and also shows a greater variability in structural design. Nevertheless, the basic morphologic feature shown in the preceding 3 cases may be discerned on careful examination of the material.

Somewhat wider streams of the small dark cells were found, splitting larger aggregations of the "parenchymal" cells (fig. 13 *B*). The latter approach closer in character to the parenchymal cell found in the mature pineal body. The arrangement of these cells is also such as is found in an older phase in the development of this organ (see fig. 7 *A*). Cellular organizations resembling other histogenetic phases are encountered in other parts of this tumor.

CASE 6.—*Pinealoma duplicating the normal structure of the pineal body in a young child (20 months), in whom a normal pineal body was also found. Signs of cerebral involvement first appeared after about eighteen months of apparently normal development.*

Gross Anatomy.—The brain is under increased tension. There is marked internal hydrocephalus. On raising the occipital lobes, a soft necrotizing tumor mass is exposed. It involves the pineal region and extends posteriorly so as to invade the vermis of the cerebellum. The pineal body is found in its normal location. In a sagittal section of the brain the tumor is found to have completely replaced the quadrigeminal plate (fig. 14 *A*).

Microscopic Anatomy.—The identification of the character of the tumor offers no difficulty and is enhanced by the striking similarity between the structure of the tumor and that of the pineal body in this case. The lobulation, the formation of fairly solid cords or irregularly round masses of cells, and the connective tissue trabeculae are present in both the pineal body and the tumor, though more marked in the pineal body (fig. 14 *B*). In other parts of the tumor there are slight structural deviations so that in one part there are two distinct zones; an area consisting of an irregular mass of the small round deep staining cells adjoins an area made up of the larger, little differentiated parenchymal cells.

CASE 7.—*Pinealoma simulating several different stages of the normal pineal body. It was found in a man 32 years old, in whom signs and symptoms of an*

expanding intracranial lesion developed. Transient amaurosis, vertigo and impaired memory marked the advance of the illness. The localizing signs were vague. Encephalographic examination did not show air in the ventricles, but ventriculographic examination showed the left lateral ventricle to be dilated and the third

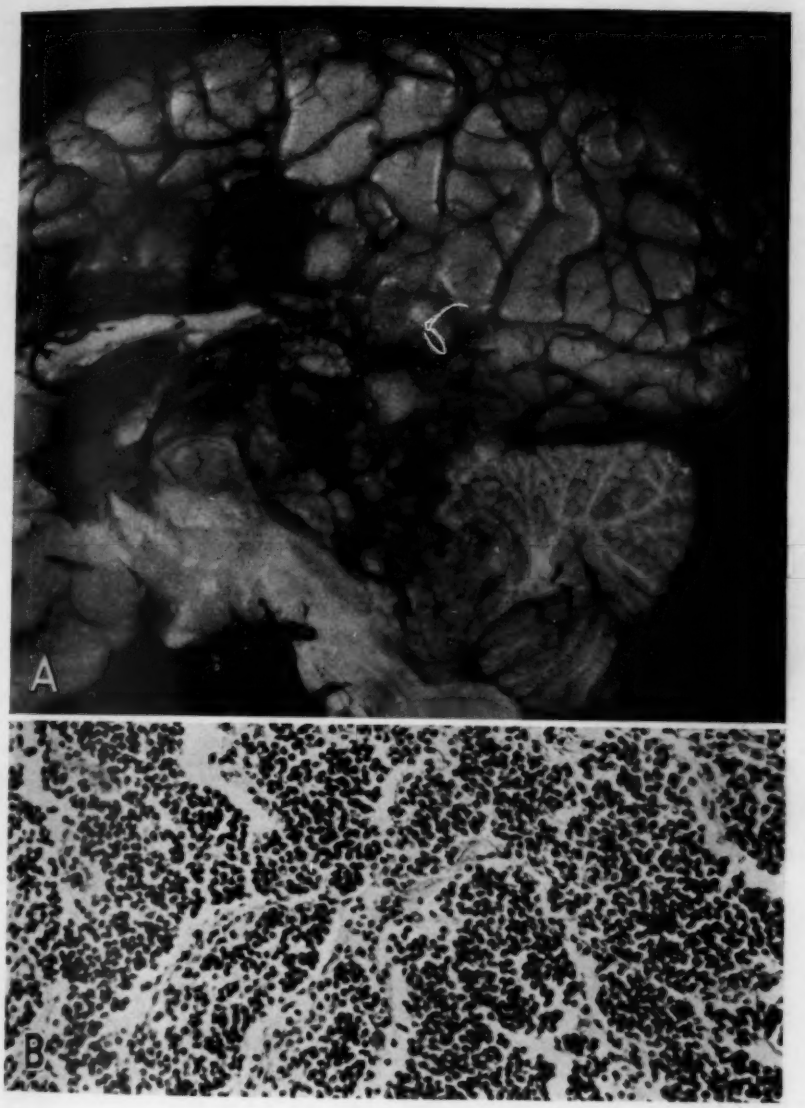

Fig. 14.—*A*, gross appearance and location of tumor (pinealoma) in case 6. *B*, section of the tumor, showing striking similarity in structure to that of the pineal body found intact in same case.

ventricle displaced to the left (the right ventricle was not visualized), and a mass was thought to be present in the floor of the left lateral ventricle. Tissue removed at craniotomy was reported as that of a primary neuroectodermal tumor, but

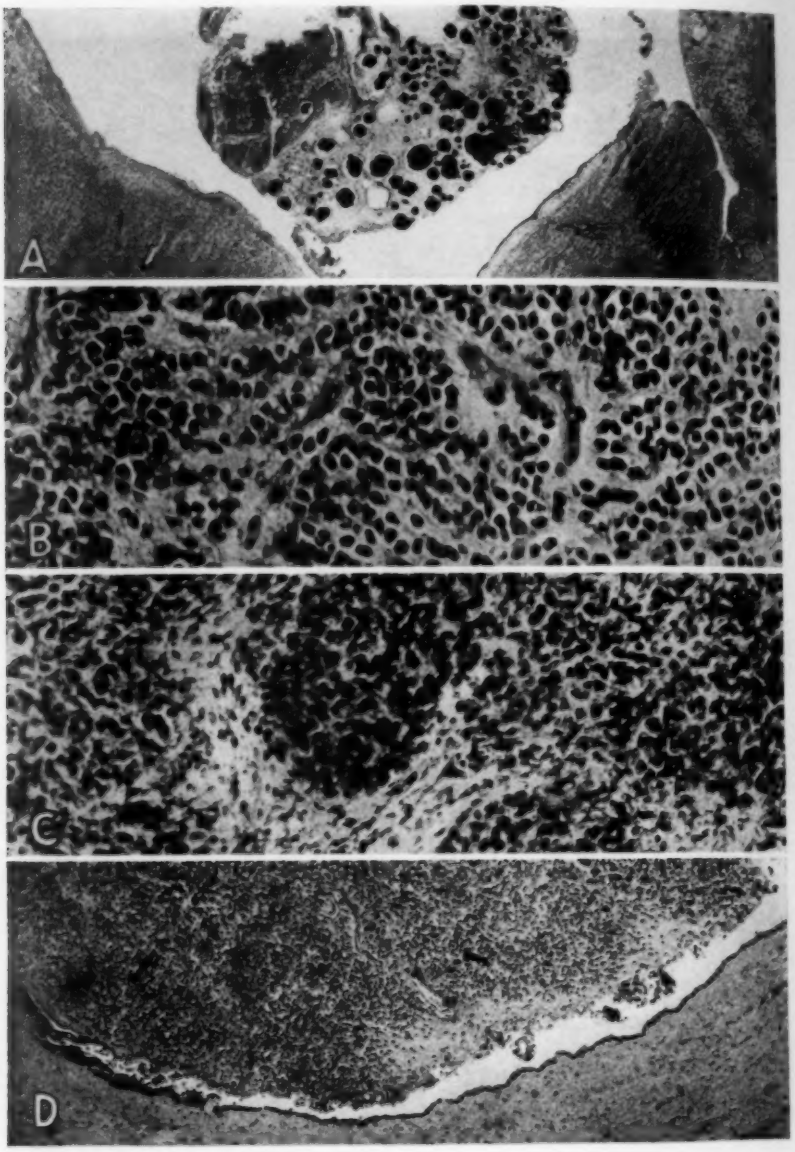

Fig. 15.—*A*, a small pinealoma situated at the posterior end and roof of the third ventricle (case 7). *B*, histologic character of tumor simulating the cell structures of the pineal anlage. *C*, lobular appearance of another part of the tumor. *D*, extension of tumor in the vicinity of the aqueduct of Sylvius.

subsequently, when the study was completed, the true character of the tumor was recognized.

Gross Anatomy.—The brain (removed through the surgical defect in two sagittally cut halves) displays in the right parietal region a lemon-sized area of softening, which extends into the basal ganglions, corpus callosum and internal capsule. The lateral ventricles are enlarged and filled with blood. The convolutions of the remainder of the brain are flattened. There is no capsule or definite line of demarcation between the diseased tissue and the surrounding tissue. The blood vessels at the base are normal. The pineal gland cannot be found.

On sectioning the brain, the following positive findings are noted: (1) an extensive symmetric internal hydrocephalus; (2) a sudden reduction in the size of the aqueduct of Sylvius directly posterior to its communication with the third ventricle; (3) some swelling and loss of markings in the most anterior portion of the mesencephalon; (4) no grossly recognizable tumor tissue anywhere; (5) a great deal of traumatic disorganization in the right hemisphere.

The dilatation of the ventricles gives the impression of an obstructive form of hydrocephalus. This observation suggests the existence of a block at the posterior end of the third ventricle or in the anterior portion of the aqueduct. Areas of discoloration are found in and about the posterior part of the lateral ventricles, but these can well be due to trauma. The aqueduct of Sylvius is found to be unusually small, suggesting a form of congenital anomaly which in itself could have caused the hydrocephalus. The question then arises whether the tumor was not in part removed at operation, as it is quite reasonable to assume that the surgeon, having made an entrance into the lateral ventricle, also made one into the third ventricle in the region of the anterior portion of the aqueduct and, having seen there a tumor, removed it, for tumor tissue was removed at operation and was provisionally diagnosed as being of neuroectodermal derivation.

Microscopic Anatomy.—The midbrain, sectioned serially, reveals a small fragment of tissue which seems to form the roof of the third ventricle at a point of its junction with the aqueduct of Sylvius (fig. 15 *A*). On higher magnification this fragment seems to consist of a mass of calcium plaques bordering on a small round cell aggregation. The latter consists of cell nests alternating with small deposits of calcium. When studied with higher power, these display a tendency to take on a pattern (fig. 15 *B*) not unlike that seen in the pineal body in its sixth month of prenatal development. In another area the lobular formation bears a strong resemblance to that seen in the developing pineal gland in its fifth month of postnatal life (fig. 15 *C*). In still another part of the tumor island, when this is traced caudally, remnants of the pineal diverticulum may be recognized (fig. 15 *D*) as extensions of the underlying aqueduct of Sylvius.

CASE 8.—*Pinealoma in a boy of 2½ years, the tumor showing an unusual extension into the left cerebral hemisphere and the third ventricle, and causing, in addition to marked internal hydrocephalus, manifestations of disturbed regulation of temperature.*

Gross Anatomy.—The brain is voluminous and fluctuating and shows marked thinning of the cerebral mantle, particularly in the posterior part of the frontal lobe of the left cerebral hemisphere, where the mantle is thinned out beyond that of the rest of the brain. At this point there is displayed a large, very vascular tumor mass. (In the process of removing the brain, the tumor disengaged itself and broke up into several fragments of very vascular material, containing scattered small islands of a grayish substance. It filled about one quarter of a 250 cc. jar.)

When the midbrain is exposed, its tectum is found to be disorganized. A grayish gelatinous substance which is found covering the quadrigeminal plate can be traced to a dilated third ventricle (fig. 16 *A*) and then into the left lateral ventricle, where the major portion of the tumor is found. The impression is gained

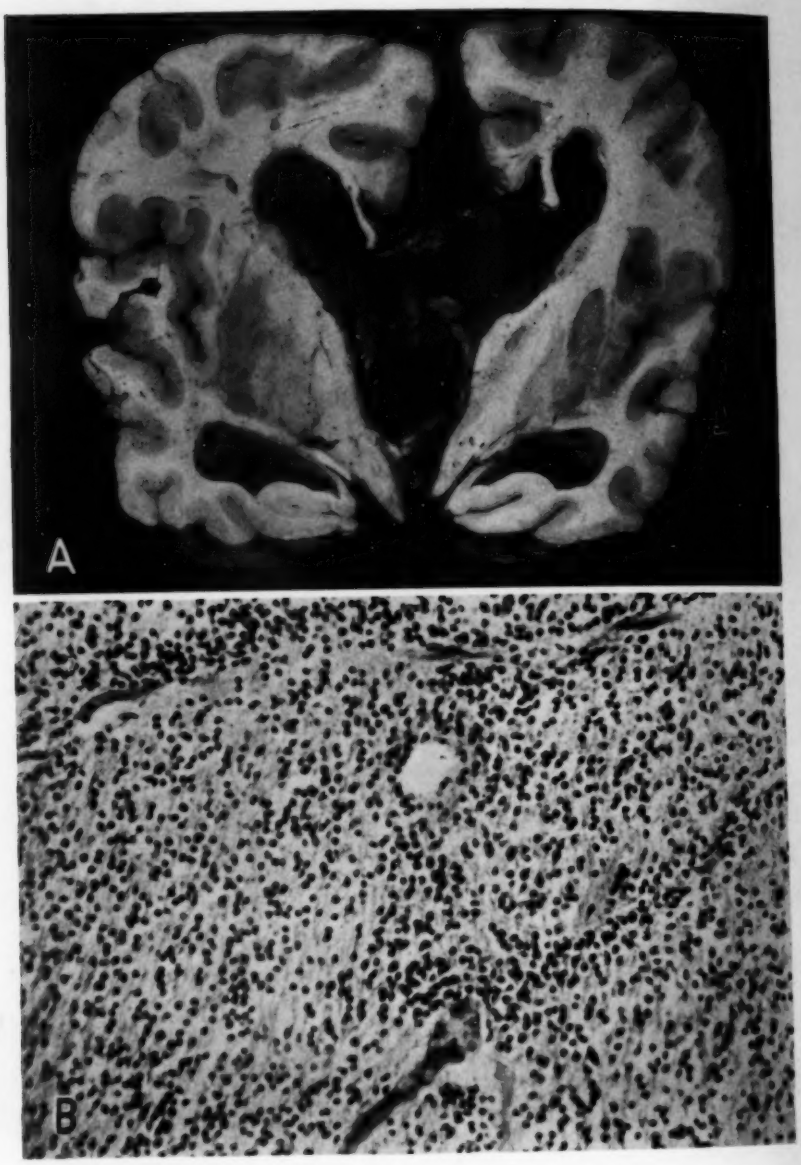

Fig. 16.—*A* and *B*, gross and microscopic appearance of the pinealoma in case 8.

that the tumor arose in the quadrigeminal plate and extended through the third ventricle into the left lateral ventricle.

On sectioning the brain, there is revealed dilatation of the ventricles in front of the aqueduct of Sylvius. The body and anterior horn of the left ventricle apparently lodged part of the tumor, as they are larger than other ventricular compartments and their walls are eroded. In the region of the posterior commissure there is found a small cystic mass, which appears to be a remnant of the tumor left attached at the point of origin. The pineal body cannot be found.

Microscopic Anatomy.—Sections taken from various parts of the tumor present a fairly uniform histologic picture. The structural design bears a strong resemblance to the cellular arrangement seen in sections of the pineal body during the very early stages of its postnatal period of development. The cells contain deeply stained, small, centrally situated nuclei with a rather large amount of cytoplasm, drawn out in short processes. The latter by joining with similar processes from adjoining cells give the impression of a syncytium. The cell type and cell arrangement are not unlike those found in neuroglia, and hence it is readily seen why a tumor of this type, particularly when found in a cerebral hemisphere, is often accepted as glioma. But failure to demonstrate typical glia cells with the aid of silver stains and the disclosure of microscopic fields with linear aggregations of round dark-staining cells, remnants of a mosaic pattern (fig. 16 *B*), recall the structure of the pineal body in an earlier histogenetic phase of the organ. Hence the diagnosis of this tumor as pinealoma is quite probable.

CASE 9.—*Pinealoma containing a large colloidal cyst and a small mass of neoplastic tissue, the latter resembling in structure a well differentiated pineal body. It completely replaced the tectum, mesencephali and invaded the left lateral ventricle. It occurred in a girl of 11 years, who presented signs and symptoms of an expanding intracranial lesion affecting the brain stem near the sylvian aqueduct.*

Gross Anatomy.—The brain is markedly flattened. On raising the occipital lobes, a soft tumor is exposed. It extends from the superior vermis to the quadrigeminal plate and beyond it. A large colloid cyst is found occupying the right lateral ventricle (fig. 17 *A*). It completely fills this ventricle as far forward as the foramen of Monro and posteriorly as far as the junction of the descending and posterior horns. It then extends backward over the roof of the aqueduct of Sylvius, where it is embedded in a depression over the lateral lobe and vermis of the cerebellum. In its posterior extent it almost completely destroyed the tectum of the midbrain and some of the tegmentum on the left side. The ventricular cavities are markedly distended. The third ventricle is widely open but is displaced to the left and deformed. The cyst has a well defined capsule, which measures about 2 mm. in thickness. It is free in its anterolateral aspect but adherent to adjacent tissues on its ventral-median aspect. It is filled with a jelly-like substance of a grayish green hue; on its ventral surface it presents a small spongy mass, cystic in some parts. These areas bear a striking resemblance in structure to the pineal body. The pineal body cannot be found.

Microscopic Anatomy.—The finer structure of this tumor shows a strong similarity to that of the adult pineal gland and suggests the diagnosis of pineal hyperplasia (fig. 17 *B*). Such a diagnosis is occasionally made. But it is rather difficult to draw a distinct line of differentiation between hyperplasia and new

growth. In this instance, because of the youth of the patient and the size of the tumor, the neoplastic character is indisputable. It is to be noted, however, that the pinealoma in this case is of the more mature character.

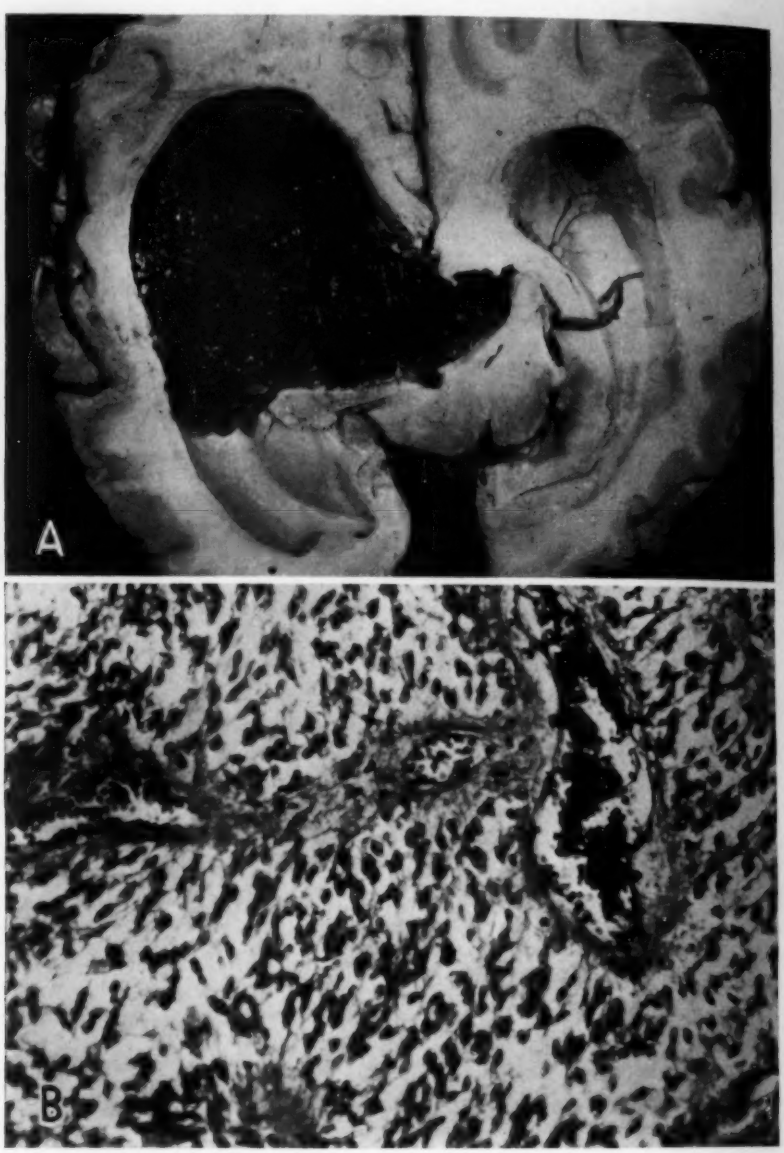

Fig. 17.—*A* and *B*, gross and microscopic appearance of the pinealoma in case 9.

CASE 10.—*Pinealoma displaying in part a structure simulating that of a well differentiated pineal body and in part cell elements and cell organization which show a departure from any of the typical stages of the histogenesis of the pineal body. The patient was a man aged 32.*

The onset of the symptoms and signs was abrupt and recent (six weeks); the symptoms were headache, persistent hiccup, obstipate constipation, humming noise in the ears and a rise in temperature. Fixed pupils, ocular palsies and nystagmus

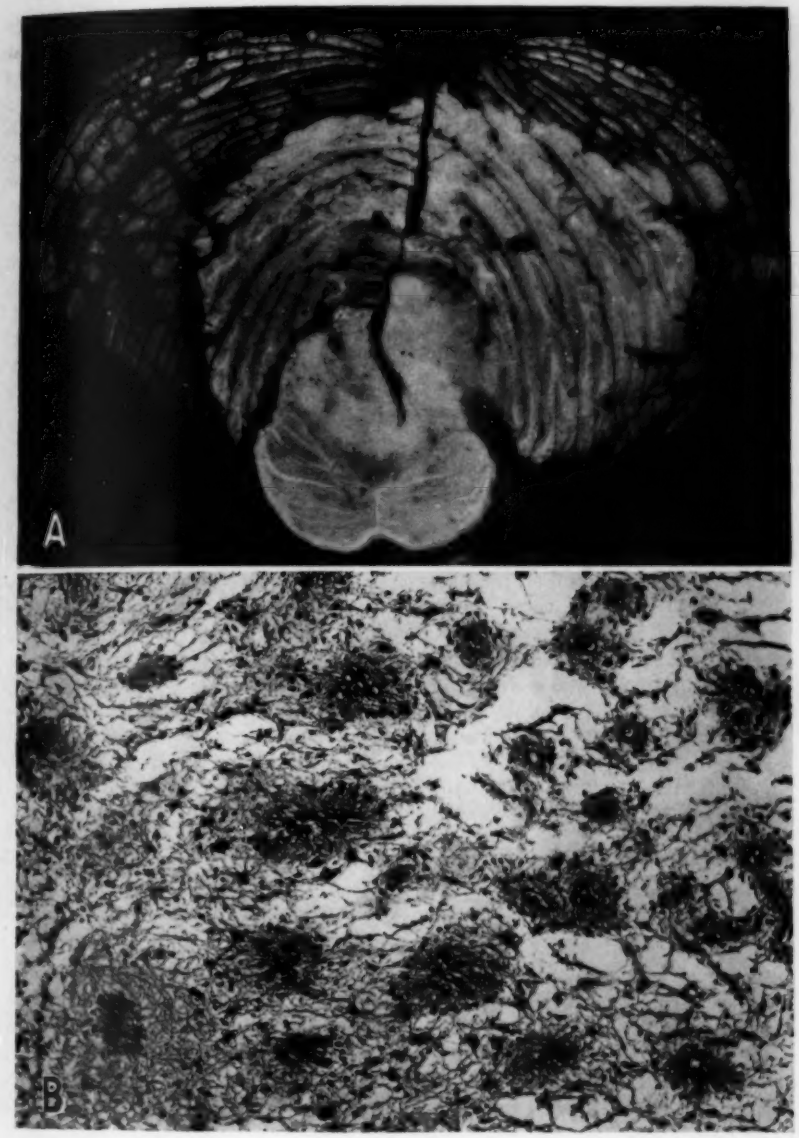

Fig. 18.—*A* and *B*, gross and microscopic appearance of the pinealoma in case 10.

were the significant focal signs. Encephalographic examination failed to disclose an appreciable amount of air in the ventricles.

Gross Anatomy.—The brain shows a deep, gaping horizontal incision in the right temporoparietal region, approximately 1½ inches (3.5 cm.) by ½ inch

(1.3 cm.). The surfaces of the brain are otherwise not abnormal. A median incision through the vermis reveals in its anterior portion and pressing into the fourth ventricle a circular tumor mass, about 1½ inches in diameter, smooth and gelatinous in appearance. On sectioning, a large necrotic channel is found in the right hemisphere, in the lower part of the middle of the parietal lobe. From there it extends on to the mesial surface of the other hemisphere up to a point about 2 cm. mesial to the lateral ventricle. A hard, nodular tumor, well circumscribed and delimited from the adjacent tissue, is found on the mesial surface of the left hemisphere. It involves the entire width of that hemisphere mesial to the posterior horn of the lateral ventricle and extends from the posterior end of the lenticular nucleus for 3 cm. The pineal body is represented by a small nodular body at the posterior end of the tumor on its mesial surface.

Further sectioning of the brain stem discloses a tumor in the roof of the aqueduct of Sylvius, extending backward as far as the roof of the entire fourth ventricle. The tumor is found mainly on the right side of the quadrigeminal plate, compressing the aqueduct of Sylvius and displacing it toward the left (fig. 18*A*). In the region of the fourth ventricle the tumor seems to have invaded part of the tegmentum of the pons. In the midportion of the midbrain the tumor seems to extend backward to the tectum into the tegmentum, particularly on the right side of the midbrain.

Microscopic Anatomy.—The identification of the histologic character of this tumor is made possible by the presence of areas in which the cell form and cell organization are highly suggestive of those seen in the adult pineal body (fig. 18*B*). Many trabeculae of connective tissue, some containing blood vessels, give it a lobulated appearance. These trabeculae are lined by cells which contain rather large, round, well stained nuclei, enveloped by faintly staining cytoplasm. The latter gives rise to processes which interlace with the processes of adjoining cells and thus form a reticulum between the trabeculae.

In other parts the tumor displays cell forms which approximate closely the appearance of quite well differentiated astrocytes. Peculiarly enough, they do not take well the specific glial stains.

Aggregations of endothelial buds or somewhat incompletely developed vessels separate the tumor in some parts from the adjacent relatively normal tissue.

CASE 11.—*Pinealoma presenting histologic features characteristic of an early developmental stage of the pineal body, in which ependymal rests are quite prominent. It was found in a man 62 years old, who gave a history of gradual development (two years) of symptoms of cerebral disease; his vision and intellect gradually became impaired, and evidence of increased intracranial tension followed. Subsequently he displayed cerebellar signs, Argyll Robertson pupils, ocular nystagmus, paralysis of upward gaze, nerve deafness and pyramidal tract signs. Encephalographic examination revealed no air in the ventricles, but ventriculographic examination showed a "huge" dilatation of both the lateral ventricles and the third ventricle, and of the anterior part of the aqueduct of Sylvius. An obstructive lesion in the aqueduct was diagnosed.*

Gross Anatomy.—The brain shows evidence of increased pressure, with the left hemisphere being somewhat larger than the right. The optic nerves, optic chiasm and optic tracts are flattened.

On sectioning of the brain, there is found an exceedingly marked internal hydrocephalus, affecting both lateral ventricles, the third ventricle and the aqueduct of Sylvius. A small mass of tumor tissue is found to be attached to the left lateral wall of the aqueduct at its anterior end. The aqueduct on being traced posteriorly is found to be markedly enlarged and completely blocked by tumor tissue, which

encroaches on the quadrigeminal plate and the dorsal part of the tegmentum (fig. 19*A*). At this site the tumor measures about 2.5 cm. in the vertical plane and about 2 cm. in the horizontal plane. Directly above this tumor and fusing with it

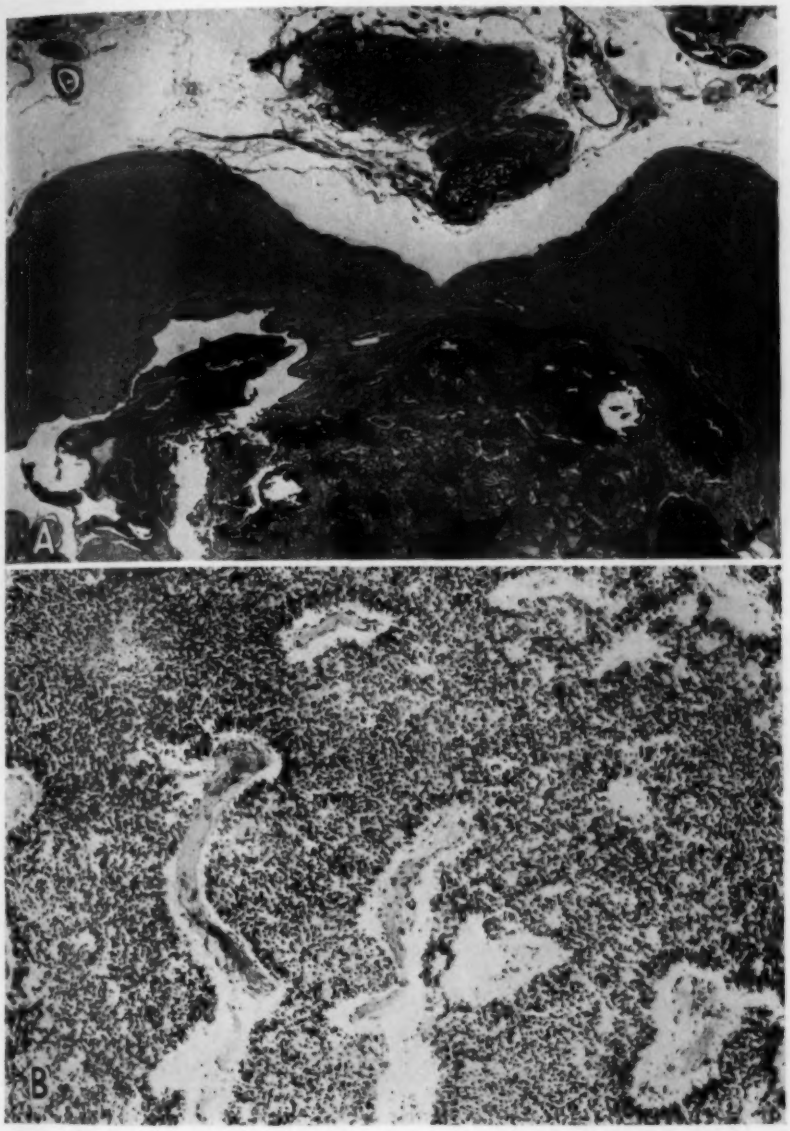

Fig. 19.—*A*, location of the pinealoma in case 11. *B*, histologic features in the pinealoma in case 11 (see text).

is the pineal body. In the left ventrolateral part of the tumor there is a small cyst filled with grayish yellow gelatinous material. Ventral to the tumor, the midbrain and pons display numerous congested blood vessels and petechial hemorrhages.

The tumor extends as far back as the transition of the aqueduct into the fourth ventricle. The upper part of the fourth ventricle shows some discoloration in its floor, due to the close proximity of the tumor. The fourth ventricle is large and almost completely occupied by a displaced vermis covered by the choroid plexus.

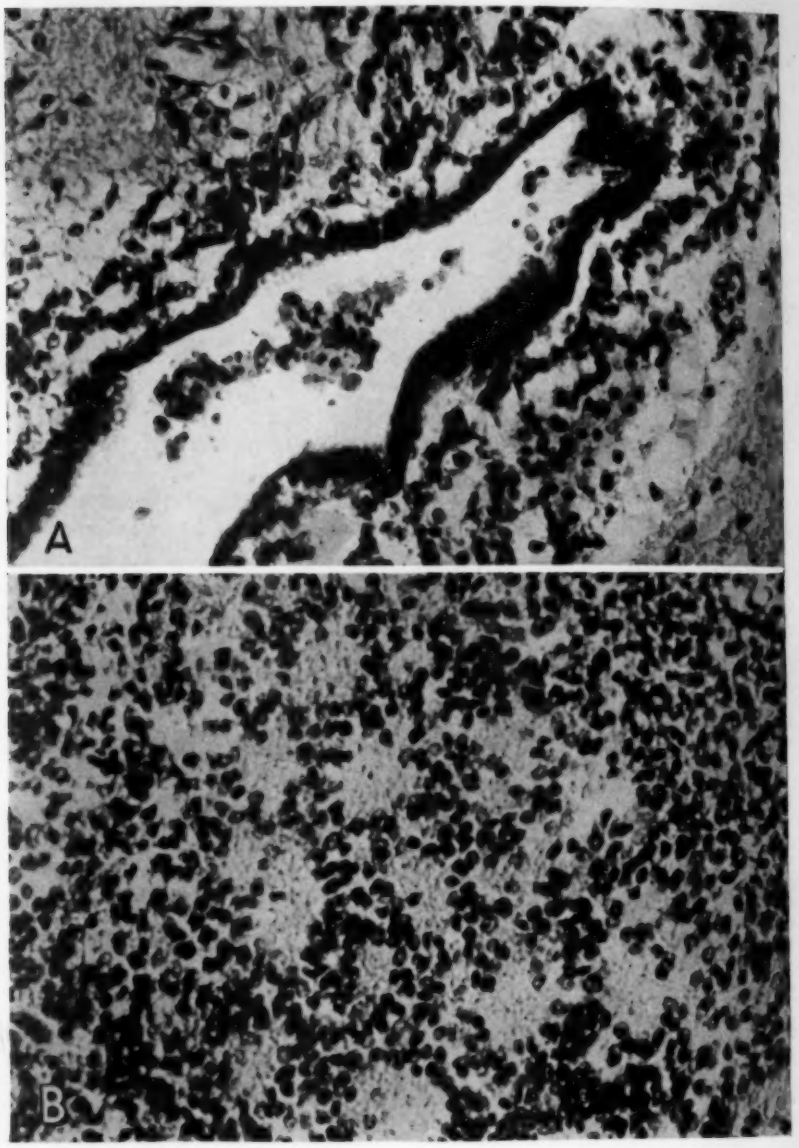

Fig. 20.—*A* and *B*, histologic features in pinealoma in case 11.

Microscopic Anatomy.—Transverse serial sections through the midbrain and attached pineal body, stained with hematoxylin-eosin, disclose two neoplastic areas, one in the pineal gland and the other in the midbrain (fig. 19 *B*). The aqueduct

is found to be completely displaced to one side, and a tumor mass occupies the ventral part of the tectum and the dorsal part of the tegmentum. It is very cellular and vascular and well demarcated from the surrounding brain tissue, which is compressed. Most of the cells are poorly differentiated and contain large nuclei, rich in chromatin. The cystoplasm is scanty. These cells are usually arranged compactly in sheets, which are interrupted by ependyma-lined cystlike structures (fig. 20*A*). Where the tumor cells are gathered about blood vessels, they bear a marked resemblance to ependyma, both in appearance and in arrangement. The cell nucleus is located at the cell pole distal to the blood vessel, while at the other pole the cytoplasm often contains vacuole-like spaces. Some of the perivascular spaces are enlarged and contain irregular refractile strands of a pink-staining substance (Mayer's mucicarmine stain discloses no mucin).

Ependyma-lined prolongations from the aqueduct may be traced for a short distance into the tumor, where the ependyma of such a channel is found to be intact and accompanied by a subependymal band of loosely arranged tumor cells (fig. 20*B*).

Along the ventral part of the pineal body the regular outline of the gland is interrupted by an oval expansion. This consists, for the most part, of deeply staining cellular tissue, clearly demarcated from normal-appearing pineal tissue and resembling that found in the tumor in the midbrain. Here, rosette-like formations are found, and ependyma-like cells are present not only about some blood vessels but also at the periphery of the tumor mass. In both tumors, the perivascular ependyma-like formations resemble structures found in the pineal bud in the fifth to sixth months of fetal life. The rosette-like structures (fig. 20*B*) resemble similar forms seen in the pineal gland during the twentieth to twenty-fifth months after birth.

Where the pineal gland reaches its greatest area in cross section, the tumor is also most extensive and, furthermore, encloses two adjoining well defined oval areas, characterized by multinucleated foreign body giant cells associated with many clearcut fusiform spaces of varying size. These spaces probably mark the site of deposition of fat-soluble crystals (cholesterol), such as are found in embryonal rests. Within the relatively normal part of the pineal gland at this level, there is an irregularly shaped space lined by ependymal cells. This space, a normal finding, represents a closed-off portion of the primitive pineal diverticulum.

CASE 12.—*Pinealoma displaying a wide departure from the typical pinealoma but linked to this variety of tumor through the medium of the earlier stages in histogenesis of the pineal body, particularly those seen in case 11. It presents as a most striking feature the presence of many monstrous giant cells, such as are seen in spongioblastoma multiforme (a gliogenous tumor form). This feature places it among pinealomas of the malignant type in which ependymal derivatives follow the gliogenous lineage of cell formation.*

The patient was a girl aged 16 years, in whom developed abruptly recurrent attacks of headaches, ringing in the ears, vomiting, dizziness, photophobia, diplopia, somnolence and retention of urine. Her signs included fixed pupils, paralysis of upward gaze and some weakness of downward gaze, nystagmoid movements of the eyes, bilateral external rectus paresis and bilateral nerve deafness. There were signs of increased intracranial tension. Ventriculographic examination showed bilateral symmetric dilatation of the lateral ventricles and enlargement of the third; the

aqueduct and the fourth ventricle were not filled with air. Roentgen therapy brought about a remarkable transient improvement, but reappearance of all signs and symptoms resulted in death.

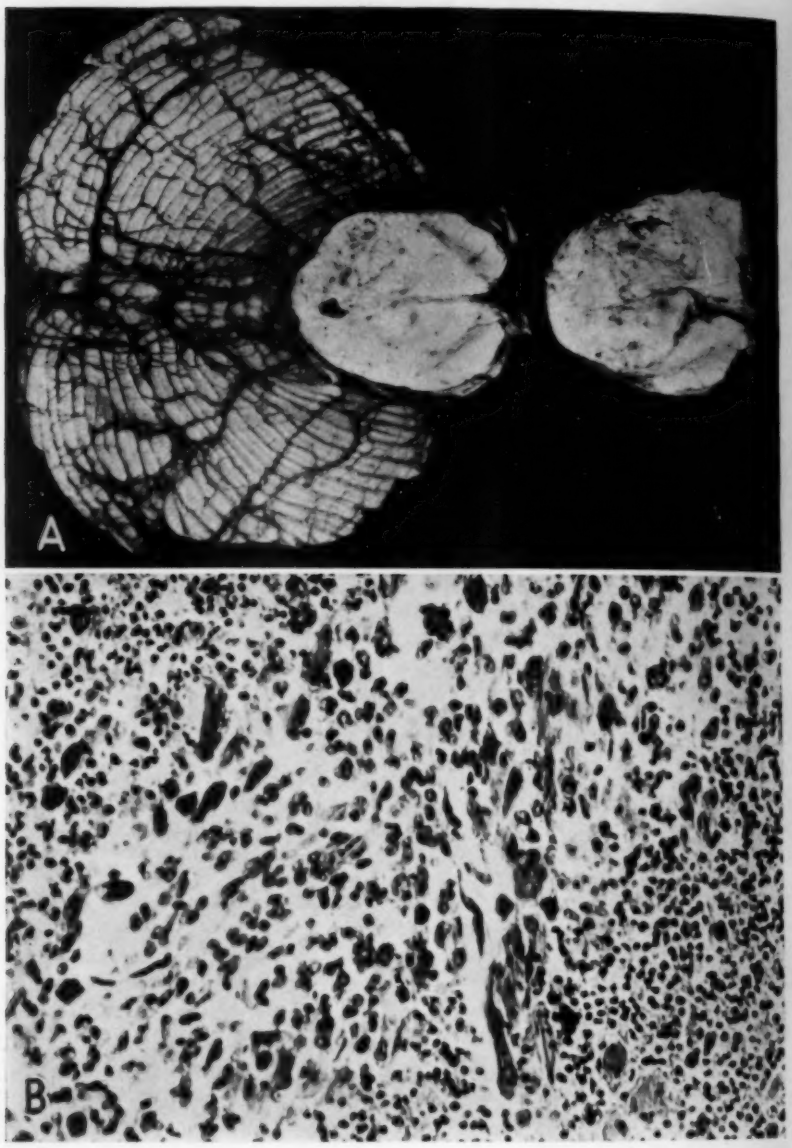

Fig. 21.—*A* and *B*, gross and microscopic appearance of the pinealoma in case 12 (see text).

Gross Anatomy.—The surfaces of the cerebral gyri are flattened. At the base of the brain there is herniation of the third ventricle, with distortion of the chiasm of the optic nerves. The pons is firm, appearing larger than normal. On separa-

tion of the hemispheres in the region of the splenium of the corpus callosum, an extremely hard mass which infiltrates the pons is noted. This mass is continuous with the structure of the pons, and no normal pineal gland can be found. The venous sinuses and vascular bed are normal.

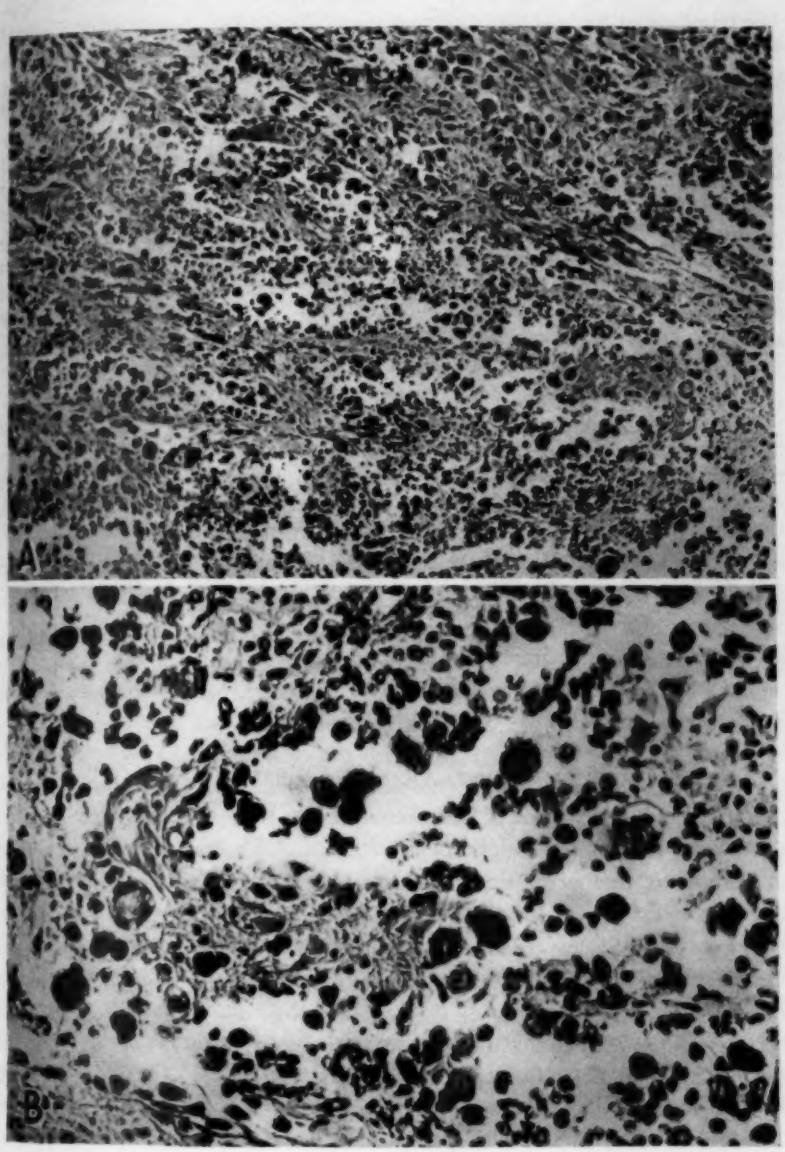

Fig. 22.—*A* and *B*, some spongioblastic features of the pinealoma in case 12 (see text).

Sectioning of the brain reveals a marked internal hydrocephalus. The left ventricle, owing to the swelling and the presence of a tumor mass in the thalamus, is smaller in size than the right. The third ventricle is narrow in its upper part above the massa intermedia. The aqueduct of Sylvius is almost completely closed at its anterior end by tumor tissue. It is fairly wide open in the rest of its course. The fourth ventricle is of about normal size. The left thalamus at about the level of the middle commissure shows a round, fairly well demarcated tumor mass occupying its dorsal two thirds and about one half of its width, leaving a narrow margin of tissue between it and the third ventricle and a wider zone lateral to it. This mass, when followed posteriorly, is found to widen out and to occupy almost the entire thalamus and to spread over to the opposite side, affecting about the medial one half of the thalamus. Further posteriorly the tumor tissue involves structures directly ventral to the aqueduct, sparing the red nucleus on the right but involving this nucleus on the left. On separating the mesencephalon from the overhanging corpus callosum, a hard mass is noted which seems to be distinct from the underlying mesencephalic tissue and gives the impression of being an enlarged pineal body. When this mass is studied further, it is found to be separated at certain points from the overlying midbrain. At its base, however, which is wide, it is adherent to, almost inseparable from, the underlying tumor tissue (fig. 21).

The pons is uninvolved. The cerebellum is also free from gross change.

Microscopic Anatomy.—In some parts of the tumor the cellular arrangement bears a strong resemblance to that seen in the more typical pinealoma. Here are recognized aggregations of small cells, bordering on areas containing large cellular elements. The latter, however, show marked deviation in size and form from the large cells seen in the typical pinealoma. They are frequently multinuclear and display a number of variations in cell form. Some of them are giantlike in size and irregular in outline; others are equally large but oval or round in outline. In other parts of the tumor the structure is strikingly different.

Here the cellular aggregations occupy narrow spaces between connective tissue partitions (fig. 22 *A*). The cells are large, most frequently multinuclear and not unlike the type seen in the malignant form of glioma, better known as spongioblastoma multiforme (fig. 22 *B*).

SUMMARY OF ANATOMIC OBSERVATIONS

In all of the 12 cases of pinealoma herein reported the quadrigeminal plate was the site of origin of the tumor. While in some the tumor appeared to be partly encapsulated, in the majority it displayed an invasive character. Bilateral symmetric hydrocephalus was found in every case. This, of course, is readily explained by the effect of the neoplasm on the underlying aqueduct of Sylvius and the resultant obstruction to the outflow of the cerebrospinal fluid. In all the cases a histologic pattern reduplicating one or another stage of the normal developing pineal body was established. In some instances a more thorough search was necessary to discover this feature; in others the admixture of ependymal derivatives in a stage of low differentiation made this problem still more difficult. However, the material in general provided sufficient evidence to establish the structural similarity and the common origin of the tumors occurring in the pineal region, in spite of the apparent divergence in their cellular structure. Dissimilar as

they appeared, it was possible to match the structure of any one of these tumors with one or another phase in the histogenesis of the normal pineal body and in this way to trace them to the common source, an embryonal rest of the pineal body.

Of particular significance in this connection is a small embryonal rest found in the vicinity of the aqueduct of Sylvius (case 4). Here both the atresia and the blastomatous remnant of the aqueduct of Sylvius in its vicinity are most probably congenital in formation. Alongside of this rest there is another that is noteworthy. It is a small pineal rest in the quadrigeminal plate, while overlying the latter there is a normal pineal body. Both were accidental discoveries, neither playing a part in the clinical picture.

SOME CLINICAL CONSIDERATIONS

The clinical manifestations are readily explained by the direct and indirect influence of the tumor on certain territorial divisions of the brain stem. The direct burden of the tumor is carried by the midbrain and its subdivisions: the quadrigeminal plate, the oculomotor nuclei, the red nucleus, the brachia conjunctiva and the cerebral peduncles including the pyramidal tracts. The indirect influence is the result of the compression of the aqueduct of Sylvius by the tumor, leading to internal hydrocephalus, which in turn causes some disturbance on the floor of the third ventricle—the seat of hypothalamic (vegetative) centers. With these two main features in mind, certain significant clinical manifestations occurring in association with pinealoma are easily understood. This is particularly true of the symptoms and signs of increased intracranial tension which were present uniformly in all instances. They included headache progressively rising in intensity, nausea, recurrent projectile vomiting, dizziness and papilledema. Diplopia was of frequent occurrence, readily explained by the effect of the tumor on the oculomotor nuclei. Argyll Robertson pupils, an almost pathognomonic sign, pointing to disruption of the quadrigeminal plate, the usual seat of the tumor, was most frequently encountered. Skew deviation is a fairly frequent finding and is often associated with involvement of the brachia conjunctiva. Pubertas praecox, a clinical feature considered for some time to be characteristic of pineal tumor was present in one case, and there not to a degree which would justify consideration of the case as a true instance of precocious development of sex character. In this connection it is rather significant that in a recent study by Bing, Globus and Simon [5] it was pointed out that the incidence of pubertas praecox in verified cases of pineal tumor is not

5. Bing, J. F.; Globus, J. H., and Simon, H.: J. Mt. Sinai Hosp. **4**:6, 1938.

as great as is usually assumed. It was also noted that disturbances of other vegetative functions are often encountered in instances of verified pineal tumor. The explanation for these occurrences was found in the hydrocephalus caused by the effect of such a tumor on the aqueduct of Sylvius. The coincident dilatation of the third ventricle results in alterations in the hypothalamus, the part of the brain stem concerned with regulation of vegetative functions. The coexistence of vegetative disorders alongside disturbances in the development of sex character suggests the probability that the normal evolution of sex character is but an expression of vegetative (visceral) organization of the organism. By shifting the control of the development of sex character from the pineal body to the hypothalamus an explanation may be found for the relative infrequency of pubertas praecox in verified cases of pinealoma.

Cerebellar signs are quite frequent and are expressed in awkward use of the extremities, as unsteadiness, wide-based gait and other forms of ataxia. The proximity of the tumor to the converging dentorubral fibers is in part responsible for these disturbances. Pyramidal tract signs are, of course, frequent. They include paresis of extremities, elevated deep reflexes and pathologic reflexes. Extrapyramidal tract signs, such as tremor, loss of associated movements and episodes of decerebrate rigidity, are encountered.

SUMMARY

Tumors of the pineal region, though often revealing divergent histologic features, can be identified as members of a single neoplastic group. This identification is made possible by tracing the pineal body through the several stages of its development and matching a typical section of the tumor under investigation with that of a developing pineal body passing through a given critical histogenetic stage. Realizing that at one stage of the histogenesis of the pineal body ependymal derivatives participate in the histogenetic process and recognizing the close relationship between ependymoblasts and spongioblasts, one may reasonably assume that pinealoma may in some instances acquire the character of the spongioblastic variety of pinealoma.

On the clinical side it may be pointed out that precocious development of sex character need not be regarded as a clinical expression of pinealoma but should be accepted as a manifestation of vegetative disturbance, resulting from hypothalamic disorder.

Among other clinical features, Argyll Robertson pupils, vegetative disorders, as somnolence, polydipsia, polyuria and ophthalmoplegias, resulting in diplopia, skew deviation, and impairment of upward gaze, when added to other evidences of increased intracranial tension point to an expanding lesion in the midbrain region and lead to a fairly secure diagnosis of pinealoma.

EFFECT OF RENAL DAMAGE ON THE TOXICITY OF HYPERVITAMINOSIS D IN RATS

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FAYETTEVILLE, ARK.

Factors which may influence the toxicity of vitamin D in the animal organism have received some attention experimentally. They have been shown to be chiefly of a dietary nature and are concerned either with vitamin interrelationships or with the calcium-phosphorus ratio of the food supply.

Thoenes,[1] as well as Gross-Selbeck,[2] succeeded in offsetting the symptoms of hypervitaminosis D in the rat by the simultaneous administration of large amounts of vitamin A–containing fat or of the vitamin A concentrate vogan. Morgan and co-workers,[3] on the basis of extensive biochemical studies, showed that a large excess of vitamin A, two hundred and fifty to one thousand times that needed under normal conditions, may decrease but does not eliminate the harmful results of an excess of viosterol. Thus, an interrelationship in the nature of an antagonism has been demonstrated between vitamins A and D, so far only on the basis of large doses.

Shelling[4] carried out a series of experiments designed to clarify the discrepancies apparent in the literature as to the toxicity of viosterol and concluded that the conflicting results could be attributed in part to differences in the character of the diets used, particularly with regard to the contents of calcium and phosphorus. He showed that in the presence of a calcifying agent (viosterol) an increase in the amount of phosphorus in the diet renders the organism more susceptible to hypercalcification. The high phosphorus–minimal calcium diet which produced this result represented a calcium-phosphorus ratio of 1 : 4.32.

From the Department of Biochemistry and the Agricultural Experiment Station, University of Arkansas.

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1. Thoenes, F.: Deutsche med. Wchnschr. **61**:2079, 1935.
2. Gross-Selbeck, C.: Klin. Wchnschr. **14**:61, 1935.
3. Morgan, A. F.; Kimmel, L., and Hawkins, N. C.: J. Biol. Chem. **120**:85, 1937.
4. Shelling, D. H.: Proc. Soc. Exper. Biol. & Med. **28**:298, 1930.

Analysis of a third factor which may aggravate the toxic effects of excessive amounts of vitamin D, namely, the role of renal injury, was stimulated by the production of arterial calcification in a group of rats chronically deficient in vitamin A. A preliminary report [5] covering the first phases of this work has already appeared.

EXPERIMENT I

Forty rats were subjected to a succession of two or more depletions of vitamin A. After each depletion except the last, recovery and a minimal storage of vitamin A were initiated by oral administration of carotene [6] for a brief period. An amount of vitamin D greatly in excess of the optimal daily requirement was incorporated in the ration [7] by irradiation of its dry components. Assays [8] of the ration revealed a content of 600 international (U. S. P. XI) units of vitamin D per gram.

Control rats, representing litter mates of the same sex and same initial weight, received 3 drops daily of carotene as the sole supplement to the irradiated ration. Throughout the generally five to seven month period of the experiment, the growth curves of the control animals remained either normal or slightly subnormal. Their allotment of food from day to day was based on the relative plane of growth, so that they ingested a greater daily average amount of vitamin D than their A-deficient litter mates.

Results.—At autopsy only the A-deficient rats showed calcification of blood vessels. Histologic studies (hematoxylin-eosin and von Kossa methods of staining) confirmed this striking difference with almost complete regularity in the 40 pairs of rats.

COMMENT

The average daily intake of food of each A-deficient rat throughout the entire experiment was approximately 10 Gm. This amount represented 6,000 international (U. S. P. XI) units of vitamin D. Since the food consumption of the control rats averaged 12 Gm. per day, a cor-

5. Opper, L.: Proc. Soc. Exper. Biol. & Med. **40**:449, 1939.

6. This was supplied by the Proctor and Gamble Corporation, Cincinnati.

7. Casein, 20; McCollum's salts no. 185, 4; Northwestern yeast (dehydrated), 10; dextrin, 51; hydrogenated cottonseed oil (Crisco), 15. The composition of McCollum's salts no. 185 is as follows:

	Gm.
NaCl	86.5
$MgSO_4$ (anhydrous)	133.0
$NaH_2PO_4.H_2O$	173.5
$Ca(H_2PO_4)_2.H_2O$	270.0
K_2HPO_4	477.0
Calcium lactate	650.0
Ferric citrate	59.0

Northwestern yeast is a brand of brewers' yeast.

8. Samples of the irradiated ration were assayed according to the pilot and U. S. P. XI methods by the Laboratory of Vitamin Technology, Chicago, through the aid of Dr. Howard J. Cannon.

respondingly larger amount of vitamin D, namely, 7,200 international (U. S. P. XI) units daily, was ingested by these rats. Nevertheless, no trace of calcification appeared in the vessels of the latter.

In an effort to account for the definitely greater susceptibility to the toxic effects of vitamin D observed in rats chronically deficient in vitamin A, certain dietary factors were considered. An analysis of the calcium-phosphorus content of the ration disclosed 0.504 mg. per hundred grams for calcium and 0.808 mg. for phosphorus, representing a ratio of 1:1.6. Although the ration was slightly high in phosphorus, this ratio indicated a balance between the two elements which is close to optimal and certainly far short of that excess of phosphorus which is required for heightened susceptibility to the toxic influence of vitamin D.[4] Moreover, both rats of each pair received the same ration, but only the test rat showed pathologic calcification.

Since protection of the controls against vitamin A deficiency consisted of 3 drops of carotene, the equivalent of 864 U. S. P. units daily, the weight of this factor was also considered. In the work of Morgan and co-workers [3] those rats which received 10,000 units of vitamin D as viosterol along with much increased vitamin A, 2,500 to 10,000 units daily, had decreased but still obvious signs of hypervitaminosis D. Thoenes [1] and Gross-Selbeck [2] reported that alleviation of hypervitaminosis D resulted from the administration of just subtoxic amounts of vitamin A. The possibility, therefore, that less than 1,000 units of vitamin A daily might neutralize the effects of excessive vitamin D appeared negligible.

That vitamin A deficiency was in some manner concerned with the frequent massive calcification of blood vessels appeared inescapable in view of the condition that this factor alone represented the difference in experimental treatment of test animals and control. A remarkable correlation between the extent of renal damage and the degree of arterial calcification in the A-deficient rats (table 1) suggested the possibility of a definite sequence in the course of the disease. The incidence of keratinizing squamous epithelium and calculi within the urogenital tract appeared related to the multiple depletions of vitamin A. The dilatation of renal tubules, scarring of renal tissue and complete atrophy of the organ when a large calculus forming a mold in the calices became impacted in the pelvis often characterized the slowly progressive destruction of functioning kidney substance. The invariable absence of calcium from the walls of the renal arterioles and from Bowman's capsule suggested, too, that the effect of vitamin D on the kidneys was less chronic and less severe than were the effects of vitamin A deficiency. The 3 rats of table 1 (22, 25, 28) whose multiple depletions were mild and

therefore unaccompanied by severe renal changes, which appear late in the course of vitamin A deficiency,[9] were the only ones which wholly escaped arterial calcification.

TABLE 1.—*Renal and Vascular Changes in Forty Vitamin A–Deficient Rats*

Rat	Depletions of Vitamin A	Duration of Experiment, Days	Degree of Vascular Injury	Pathologic Changes in Urinary Tracts
1 ♂	2	115	+++	Chronic interstitial nephritis
2 ♂	2	127	+++	Chronic interstitial nephritis
3 ♂	3	131	+++	Chronic interstitial nephritis
4 ♂	3	157	+++	Chronic interstitial nephritis
5 ♀	3	158	+++	Chronic interstitial nephritis
6 ♂	3	178	+++	Chronic interstitial nephritis
7 ♀	3	206	+++	Chronic interstitial nephritis
8 ♀	3	214	+++	Chronic interstitial nephritis
9 ♀	4	230	++++	Chronic interstitial nephritis; renal atrophy (right)
10 ♀	2	141	+	Bladder calculi; pyelonephritis (bilateral)
11 ♀	2	141	++	Bladder calculi; pyelonephritis (bilateral); chronic interstitial nephritis
12 ♀	2	151	+++	Renal calculi (bilateral); chronic interstitial nephritis
13 ♂	3	180	+++	Chronic interstitial nephritis
14 ♀	2	199	++	Bladder calculi; renal calculi; pyelonephritis (bilateral)
15 ♀	2	210	+++	Renal calculi (right); ureteral calculi (left); chronic interstitial nephritis
16 ♀	2	211	+	Bladder calculi; chronic interstitial nephritis
17 ♀	3	242	+++	Renal calculi (bilateral); renal atrophy (bilateral); chronic interstitial nephritis
18 ♂	2	137	++	Renal calculi (bilateral); chronic interstitial nephritis
19 ♀	2	144	++++	Bladder calculi; renal calculi (right); chronic interstitial nephritis
20 ♀	3	150	++++	Bladder calculi; renal calculi (bilateral); hydronephrosis (bilateral); chronic interstitial nephritis
21 ♂	2	155	+	Bladder calculi; hydronephrosis (bilateral)
22 ♂	2	164	0	None
23 ♀	3	179	++++	Renal calculi (right); renal atrophy (right); chronic interstitial nephritis
24 ♂	3	197	+	Chronic interstitial nephritis
25 ♂	3	197	0	None
26 ♂	3	201	+	Bladder calculi; hydronephrosis
27 ♀	3	238	++++	Chronic interstitial nephritis
28 ♂	2	145	0	None
29 ♂	2	145	+	Bladder calculi; chronic interstitial nephritis
30 ♀	2	161	++++	Renal calculi (right); chronic interstitial nephritis
31 ♂	2	225	+++	Renal calculi (right); pyelonephritis (bilateral)
32 ♂	2	225	+++	Renal calculi (right); renal atrophy (right); chronic interstitial nephritis
33 ♀	4	326	++++	Renal calculi (bilateral); chronic interstitial nephritis
34 ♀	2	275	+++	Renal calculi (right); renal atrophy (right); chronic interstitial nephritis
35 ♀	2	127	++	Chronic interstitial nephritis
36 ♂	2	130	+	Bladder calculi; renal calculi (right); chronic interstitial nephritis
37 ♀	2	177	+++	Renal calculi (bilateral); chronic interstitial nephritis
38 ♀	3	186	++++	Chronic interstitial nephritis
39 ♀	2	191	+++	Renal calculi (bilateral); chronic interstitial nephritis
40 ♂	3	192	++++	Renal calculi (bilateral); chronic interstitial nephritis

To determine whether renal damage provided the mechanism whereby arterial calcification occurred in the presence of an excess of vitamin D, a second experiment was devised.

9. Wolbach, S. B.: J. A. M. A. **108**:7, 1937.

EXPERIMENT II

Twenty-seven young rats of both sexes were provided with the same ration used in experiment I, unirradiated to insure complete absence of vitamin D, and subjected to partial nephrectomy. The method of reducing substantially the amount of functioning renal tissue was essentially that used by Chanutin and Ferris.[10] The left kidney was removed under ether anesthesia when the animal weighed from 50 to 80 Gm. In a second operation, following the first by approximately one week, the upper half of the right kidney was ligated. After a lapse of another week, when recovery and good growth were assured, a solution of viosterol [11] in corn oil was administered by dropper almost daily to the partially nephrectomized rats and their litter mate controls. Whenever the weights of the animals which had been operated on lagged behind those of the controls, the dosage of vitamin D for the latter was adjusted accordingly, so that, as may be seen in table 2, their average allotment of viosterol always exceeded that in the corresponding test animals to a varying degree.

As soon as symptoms of marked toxicity appeared in the nephrectomized litter mate, the pair of rats were killed and examined. An effort was made to vary the number and size of the doses of vitamin D in the nephrectomized litter mates. Symptoms of hypervitaminosis were apparent in only a few of the control rats despite the larger intake of vitamin D. These symptoms were always mild, such as slight loss of weight and anorexia.

The animals were killed by bleeding from the femoral vessels, and the recovered serum was used for determinations of calcium and inorganic phosphorus according to the methods of Clark and Collip [12] and Fiske and Subbarow,[13] respectively.

Results.—Gross examination of the tissues of the nephrectomized rats revealed deposits of calcium in the aorta and its major arterial branches as well as in the cardiac muscle. The extent of vascular calcification varied from linear streaks confined to the aortic arch to diffuse calcification of the entire arterial system. The plaques of calcium in the muscle of the heart were sometimes massive. Calcification of the wall of the gastrointestinal tract and marked grittiness of the hypertrophied rest of the right kidney completed the gross pathologic picture of the partially nephrectomized rats. The controls showed only involvement of renal tissue, evidenced chiefly in the moderate calcification of the corticomedullary border.

Blocks of heart, aorta and kidney were placed in Bouin's solution for staining with hematoxylin-eosin and in 95 per cent alcohol for staining by von Kossa's method for calcium. Histologic study established the complete absence of calcium from the vascular tissue of the control rats, as well as the presence of calcium in a distribution quite comparable to that observed in the vitamin A-deficient group of experiment I in the arteries of the partially nephrectomized rats. The renal rest showed not only calcification of tubules and glomeruli but also extensive interstitial fibrosis. Calcium in the kidneys of the control rats was confined to the lumens and epithelium of relatively few collecting tubules. No histologic basis for functional damage of these kidneys was apparent.

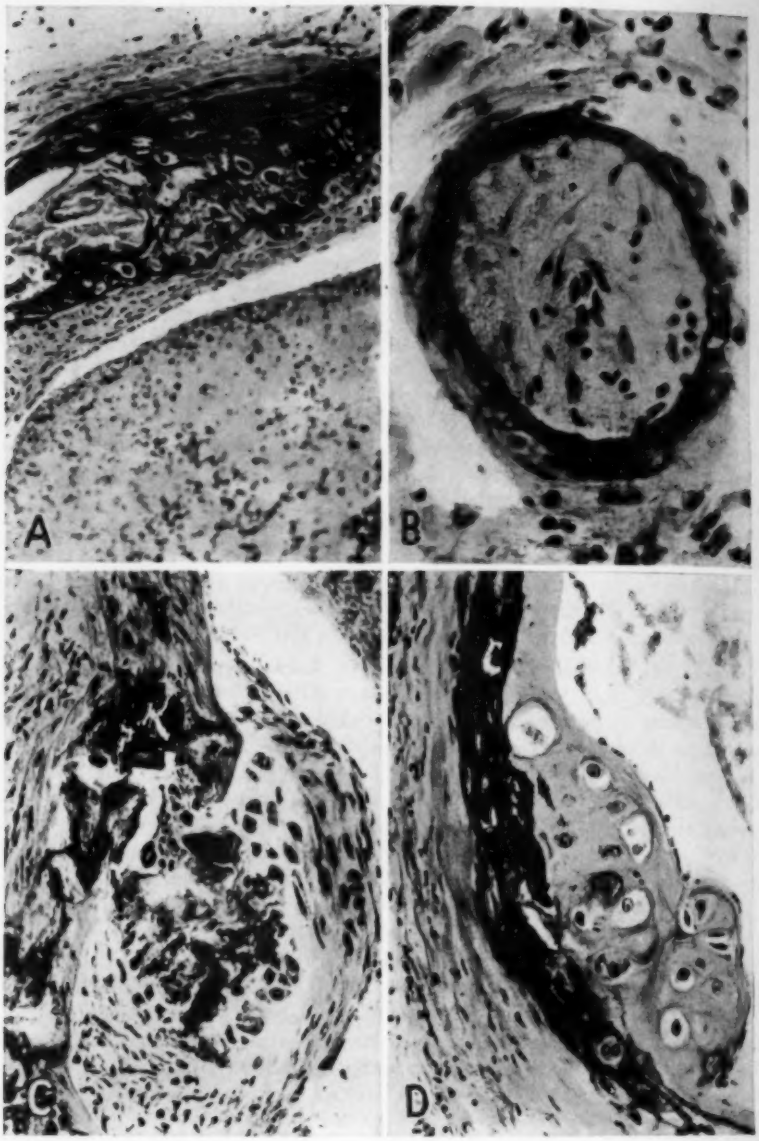
The levels of the total serum calcium of the partially nephrectomized rats and their controls were moderately elevated, with a slightly greater average increase

10. Chanutin, A., and Ferris, E. B.: Arch. Int. Med. **49**:767, 1932.

11. This was furnished by Mead Johnson & Company through the aid of Dr. Charles E. Bills.

12. Clark, E. P., and Collip, J. B.: J. Biol. Chem. **63**:461, 1925.

13. Fiske, C. H., and Subbarow, Y.: J. Biol. Chem. **66**:375, 1925.



A, section of abdominal part of aorta showing extensive medial calcification, medial necrosis and subendothelial proliferation of fibroblasts. Hematoxylin and eosin stain; × 200. *B*, branch of coronary artery showing circular medial calcification and occlusion of the lumen by subendothelial proliferation of fibrous connective tissue. Hematoxylin and eosin stain; × 475. *C*, carotid artery showing advanced organization of the necrotic and calcified wall. Note the protrusion of the reparative process into the vascular lumen. Hematoxylin and eosin stain; × 200. *D*, pulmonary artery showing dense medial calcification and a subintimal bulbous cluster of cartilage-like cells. Hematoxylin and eosin stain; × 200.

apparent in the former (table 2). The difference in the inorganic serum phosphate of each pair, with but a single exception (rat 4450), was, however, very marked. Although the serum phosphate of the control was in general moderately increased, that of the partially nephrectomized rat was frequently more than double the normal.[14]

TABLE 2.—*Comparative Values of Calcium and Phosphorus in Serum of Partially Nephrectomized and Non-Nephrectomized Rats Fed Viosterol*

		Partially Nephrectomized Rat			Control Rat		
Rat	Days of Viosterol	Average Dose of Viosterol, International (U.S.P. XI) Units	Serum Phosphorus, Mg. per 100 Cc.	Serum Calcium, Mg. per 100 Cc.	Average Dose of Viosterol, International (U.S.P. XI) Units	Serum Phosphorus, Mg. per 100 Cc.	Serum Calcium, Mg. per 100 Cc.
1 ♂	12	14,000	34.0	13.9	20,000	11.4	13.0
2 ♀	19	18,000	15.75	14.2	22,000	12.0	11.2
3 ♀	18	15,000	10.75	13.5	19,000	12.65	12.8
4 ♀	6	13,000	33.75	13.8	18,000	12.75	14.2
5 ♀	10	15,000	16.75	16.7	16,000	13.25	13.6
6 ♀	10	15,000	25.0	11.7	21,000	13.0	11.3
7 ♂	19	16,000	15.0	15.9	20,000	12.0	14.5
8 ♂	11	13,000	19.25	13.8	17,000	12.5	13.5
9 ♂	9	13,000	33.25	13.6	19,000	14.0	13.8
10 ♀	8	14,000	27.0	14.0	19,000	13.25	14.3
11 ♂	14	18,000	12.25	14.0	22,000	10.75	12.8
12 ♀	18	18,000	18.5	12.6	21,000	11.75	13.4
13 ♀	12	19,000	23.5	11.5	23,000	11.75	11.5
14 ♂	9	14,000	22.5	12.4	16,000	11.75	13.2
15 ♀	16	17,000	13.0	13.8	23,000	12.0	14.0
16 ♂	7	14,000	21.75	14.9	20,000	13.25	12.2
17 ♂	7	14,000	18.5	14.2	15,000	13.25	13.4
18 ♂	7	13,000	30.25	13.6	21,000	14.0	11.7
19 ♂	6	17,000	22.5	12.8	22,000	12.5	12.2
20 ♀	7	14,000	16.75	12.8	17,000	15.0	12.4
21 ♀	10	14,000	13.75	13.4	19,000	12.5	13.4
22 ♀	10	15,000	14.5	15.1	19,000	11.5	15.7
23 ♂	9	15,000	17.75	14.7	19,000	11.75	15.1
24 ♂	5	27,000	18.75	13.6	28,000	14.5	14.0
25 ♂	7	28,000	19.6	13.7	34,000	12.0	13.6
26 ♀	8	25,000	23.25	14.01	29,000	11.25	15.9
27 ♀	6	23,000	20.5	13.8	29,000	12.0	14.2
Total average	10	16,700	20.67	13.77	21,100	12.52	13.36

COMMENT

A few references to the work of previous investigators concerned with the production of hypervitaminosis D in the absence of initial renal damage serve to emphasize further the role of primary renal insufficiency in accelerating this condition. In the hands of different workers the amount of vitamin D in one form or another necessary to produce symptoms of toxicity in the rat has varied within wide limits. Most of this work was done before the international standard became available. Light, Miller and Frey [15] reported that 10,000 × overdosage of viosterol had no effect on the growth of rats during a period of six months. A "daily curative dosage," defined as approximately ½ U. S. P. unit of

14. The normal value for inorganic serum phosphate with the diet used is 11.0 mg. per hundred cubic centimeters of blood. This is close to the mean value of 10.3 mg. designated for the rat by C. L. A. Schmidt and D. M. Greenberg (Physiol. Rev. **15**:297, 1935).

15. Light, R. F.; Miller, G. E., and Frey, C. N.: J. Biol. Chem. **84**:487, 1929.

vitamin D,[16] would indicate that these workers found a daily dosage of 5,000 international (U. S. P. XI) units entirely nontoxic. In their long time experiments Bills and Wirick[17] found that 4,000 × overdosage of viosterol was definitely injurious. Converted into international units this amount of vitamin D is taken to represent about 10,000 international (U. S. P. XI) units per gram of diet.[18] More recently Harris, Ross and Bunker[19] were able to produce calcification of soft tissues and death within a month by feeding 50,000 U. S. P. units of vitamin D in the form of viosterol or of tuna liver oil, whereas in a second group of rats no histologic evidence of hypervitaminosis occurred as the result of the administration of 16,000 U. S. P. units daily of either substance over a period of twenty days.

The influence of phosphate on pathologic calcification has been indicated by Smith and Elvove[20] in their experiments with viosterol poisoning in rabbits. A rise in serum phosphate accompanied by only a slight increase in serum calcium generally resulted in great calcification of soft tissues. On the other hand, no abnormal deposition of calcium was found in any case in which the serum phosphate was low, even when this condition was combined with pronounced hypercalcemia. The importance which Shelling[4] attributes to a high phosphorus ration has already been alluded to. He showed further that increased phosphorus retention characterized the metastatic calcification observed with hypocalcemia in parathyroidectomized dogs.[21]

Although it is well known that an increase of inorganic serum phosphate is a sign of failure of renal function,[22] the relation of phosphate retention to the calcification occasionally noted in nephritis has not been accurately determined.[23] In the vitamin A–deficient rats of experiment I, it is likely that constant exposure to a moderate excess of vitamin D resulted in arterial calcification mainly because successive depletions of vitamin A produce progressive destruction of renal tissue to the point of insufficiency and retention of phosphate. Even when this influence is absent, it is maintained,[24] degeneration of the cells of the kidney is the

16. Frey, C. N: Personal communication to the author.

17. Bills, C. E., and Wirick, A. M.: J. Biol. Chem. **86**:117, 1930.

18. Bills, C. E.: Personal communication to the author.

19. Harris, R. S.; Ross, B. D., and Bunker, J. W. M.: Am. J. Digest. Dis. **6**:81, 1939.

20. Smith, M. I., and Elvove, E.: Pub. Health Rep. **44**:1245, 1929.

21. Shelling, D. H.: Proc. Soc. Exper. Biol. & Med. **28**:301, 1930.

22. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, Williams & Wilkins Company, 1931, vol. 1, p. 1130.

23. Barr, D. P.: Physiol. Rev. **12**:593, 1932.

24. Reed, C. I.; Dillman, L. M.; Thacker, E. A., and Klein, R. I.: J. Nutrition **6**:371, 1933.

most constant early change following excessive doses of viosterol, and this probably precedes the deposition of calcium. To Smythe and Goldman [25] "this observation is of peculiar significance not only with regard to the whole matter of calcinosis, but also with regard to sclerosis of the arteries and kidneys with less generalized calcinosis, such as is seen in the reported cases of chronic diffuse nephritis."

SUMMARY

Of 40 rats which were made markedly deficient in vitamin A several times in the course of an average period of four to seven months on a diet which included an average daily intake of 6,000 international (U. S. P. XI) units of vitamin D, 37 showed varying degrees of arterial calcification at autopsy. The extent of the calcification in each animal was commensurate with the amount of renal damage and this, in turn, was due primarily to the successive depletions of vitamin A. Since the control rats, not deficient in vitamin A but otherwise consuming the same diet, showed no traces of arterial calcification, it appeared worth while to investigate the role of renal insufficiency in potentiating the toxicity of vitamin D in rats.

To this end, 27 young rats were partially nephrectomized by complete removal of one kidney and ligation of the upper half of the other kidney in two successive operations. The effect of 13,000 to 29,000 international (U. S. P. XI) units of vitamin D in the form of viosterol administered daily over a period of six to nineteen days was determined in these animals and their controls (rats not operated on). Despite the latter's larger, average daily supplement of vitamin D, no calcification of tissues was revealed in stained sections.

The blood serum removed at autopsy from the second group of 27 rats and their controls was examined for inorganic phosphorus and for calcium. Whereas the nephrectomized rats showed a marked average elevation of serum phosphorus as compared with the controls (20.67 mg. and 12.52 mg. per hundred cubic centimeters of blood), the serum calcium in both rats of each pair was only slightly higher than normal and in approximately the same degree (13.77 mg. and 13.36 mg. per hundred cubic centimeters of blood).

It thus appears that among the several factors which may influence the toxicity of excessive vitamin D in the diet renal damage, whether it is produced by chronic vitamin A deficiency or by partial nephrectomy, may be of importance. The probable underlying mechanism is well recognized. More significant even than high serum calcium in the calcification of soft tissues is high serum phosphorus, and one of the causes of elevation in the inorganic phosphorus of the blood is renal insufficiency.

25. Smythe, F. S., and Goldman, L.: Am. J. Dis. Child. **48**:596, 1934.

RETICULUM CELL LYMPHOSARCOMA IN RATS

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Although the literature contains numerous reports of the occurrence of lymphoblastoma in general and of lymphosarcoma in particular in mice, little has been said about these conditions in rats. In the Division of Pharmacology of the Food and Drug Administration a number of experiments on chronic toxicity, carried out on Osborn-Mendel albino rats, have recently been terminated. The rats were from 24½ to 26 months (about 745 to 790 days) of age at the time of death. About 22 per cent of these old rats have shown a reticulum cell lymphosarcoma having its origin in the lymphocytic accumulations so commonly found around the large bronchi. A much smaller percentage of similar rats from 15 to 24 months of age have shown a similar tumor; the ages of the 5 tumor-bearing rats in this group were 15, 15, 20, 23 and 24 months; rats of an earlier age have not been found to harbor this tumor (table 1).

GROSS APPEARANCE OF THE LYMPHOSARCOMA

The earliest stage observed was a whitish peribronchial thickening near the hilus of the lung, more frequently that of the right lung, softer and whiter than the common peribronchial fibrosis of old rats. This small area of tumor then grew both peripherally and into the other pulmonary lobes and into the mediastinum. In some animals in which there were several discrete foci of tumor, either multicentric origin or early intrapulmonary metastasis was evident. In the cases in which the tumor was more advanced a large portion of one or both lungs and the mediastinum (table 2) were usually involved (figs. 1 *A* and *B*). In no case could we make out an origin in the mediastinal lymph nodes or in the thymus. In general, the larger the tumor masses, the softer they were. Necrosis or other degenerative change was not seen. Pleural adhesions were frequent when the growth was advanced, although the tumor showed no tendency to invade the chest wall or the diaphragm.

Metastases were seen in 6 of the 35 tumor-bearing rats. In 2 animals they were limited to the mediastinal lymph nodes and were detected only

From the Division of Pharmacology, Food and Drug Administration, Federal Security Agency.

on microscopic examination. In a third animal one kidney contained a single 5 mm. spherical tumor nodule and the other kidney a 2 mm. nodule. In a fourth animal a 2 cm. mass of tumor surrounded the colon, and there was a 2 cm. mass in the root of the mesentery, as well as mediastinal and pulmonary masses of similar size; in this animal an abdominal origin of the tumor might be considered, but the evidence for a thoracic origin is at least as good. In the fifth and sixth rats, in addition to the thoracic tumor, there were several tumor nodules up to 1.5 cm. in diameter in the mesentery; the same comment concerning the primary tumor applies here.

TABLE 1.—*Pulmonary Lymphosarcoma in Rats*

Age	Rats Examined	Rats with Lymphosarcoma	
		Number	Percentage
Over 24 months	135	30	22.2
19 to 24 months	47	3	6.4
13 to 18 months	42	2	4.8
7 to 12 months	45	0	0
1 to 6 months	54	0	0
All ages	323	35	10.8

TABLE 2.—*Mediastinal Invasion and Metastasis of Pulmonary Lymphosarcoma in Rats*

Stage of Tumor in Lung	Number of Tumors	Number with Mediastinal Invasion	Number with Metastases
Moderately or markedly advanced	25	22	6
Early but definite	10	1	0
Questionable (very early; not included)	3	0	0

All of the tumors giving rise to metastases were in animals over 24 months of age with a single exception; this was seen in a rat 24 months of age. Had the rats been allowed to live as long as possible, there might have been a fairly high percentage with metastases.

The spleen showed no gross involvement by tumor and no enlargement beyond a slight degree, seen as often in animals without tumor. Males and females were affected with about equal frequency.

MICROSCOPIC APPEARANCE OF THE LYMPHOSARCOMA

The earliest stage observable microscopically in the sections stained with hematoxylin and eosin was focal hypertrophy and hyperplasia, evidenced by an increased number of mitoses, of the peribronchial lymphoid accumulations near the hili of the lungs. The cells of the lymphoid

reticulum took much the greatest part; small round lymphocytes were present in small numbers. The resulting tumor cells were of moderate to large size and were both polygonal and rounded in outline; the nuclei were round, oval or reniform, with decreasing frequency in the order named; the cytoplasm was neutrophilic to acidophilic, small to moderate in amount and without special features; the nucleoli were of moderate

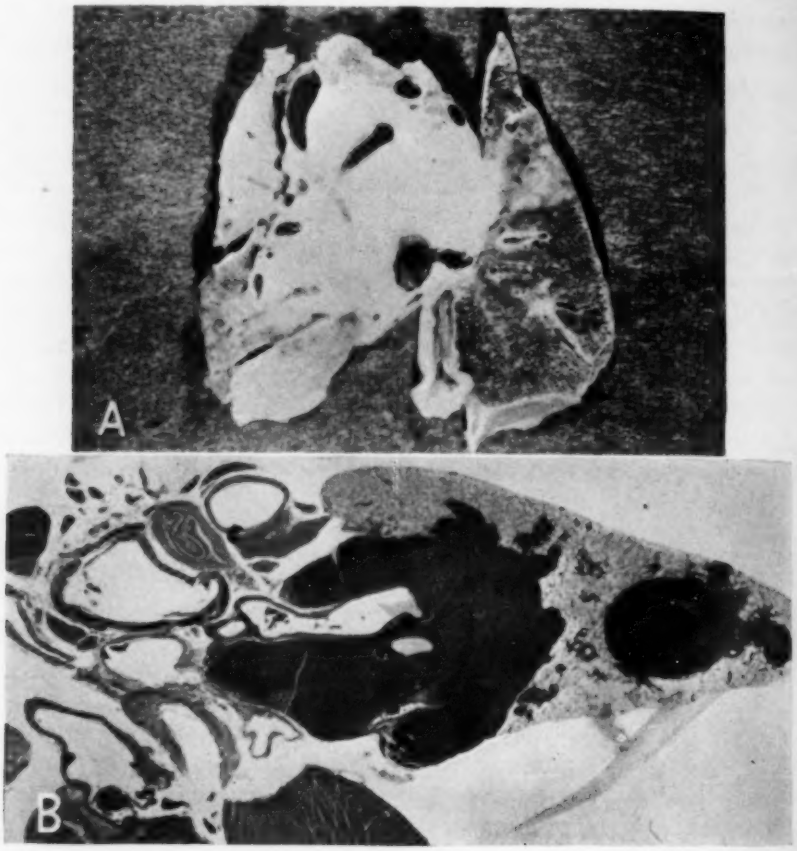

Fig. 1.—*A*, frontal section through the lungs and mediastinum of rat 130, showing lymphosarcoma in an advanced stage. The tumor involves about two thirds of the right lung and is beginning to invade the left lung. There is a large mediastinal mass of tumor. *B*, section from rat 108, showing lymphosarcoma in a moderately advanced stage. Two large areas of tumor are seen in the lung; there is early invasion of the mediastinum. Part of the heart is below.

size, usually single and occasionally multiple; mitoses were generally frequent. Occasionally, large cells with lobulated nuclei of the Sternberg-Reed type were present; in a few tumor areas macrophages containing

nuclear fragments were present in moderate numbers. Necrosis was rarely seen. The amount of fibrous stroma varied from little to much; most of it was preexistent and not produced by the tumor.

Silver impregnation of reticulum was done on 6 of our 35 tumors. In the larger tumor masses, where the cells were not growing in a rather dense mass of preexisting collagenous and reticular fibers, argentophilic reticulum was usually moderate in amount and consisted chiefly of strands radiating out from capillaries and larger blood vessels (fig. 2 *A*). In a minority of areas there was an abundant fine meshwork between the individual tumor cells.

In the more advanced stages infiltration and more or less obliteration of the walls of bronchi and large pulmonary veins (fig. 2 *B*) were frequent, and masses of tumor cells were occasionally seen in the lumens of veins. The walls of pulmonary arteries were never invaded; the cardiac atria were often involved. Mediastinal nerves were sometimes invaded.

The microscopic appearance of the metastatic growths was the same as that of the primary growth.

The mediastinal lymph nodes and the thymus were uniformly uninvolved as far as lymphosarcoma was concerned, with the exception of 2 animals in which the slightly enlarged mediastinal nodes showed areas of lymphosarcoma. Often the microscopic sections would show lymphosarcoma in the mediastinum, a direct extension of the pulmonary tumor, in close proximity to entirely tumor-free and otherwise essentially normal lymph nodes or thymus. The spleen and bone marrow showed no lymphosarcomatous, leukemic or other microscopic changes as compared with the spleen and bone marrow of animals without tumor. No lymphatic leukemia has been seen in any of our rats; about 6 have shown myeloid leukemia but none in connection with the tumor described in this paper.

POSSIBLE RELATION OF LYMPHOSARCOMA TO PULMONARY INFECTIONS

Purulent bronchiectasis has been present in many of our older rats, just as in most rat colonies.[1] In our series of 35 rats with lymphosarcoma, purulent bronchiectatic areas were noted in 11, and in some of these the walls of the ragged pus-filled cavities were formed by lymphosarcomatous tissue. However, bronchiectatic cavities were as frequent in rats without lymphosarcoma. In some rats in which purulent bronchiectasis was not present there were foci of chronic pneumonia.

1. Meyer, K. F.: Communicable Diseases of Laboratory Animals, in Jordan, E. O., and Falk, I. S.: The Newer Knowledge of Bacteriology and Immunology, Chicago, University of Chicago Press, 1928.

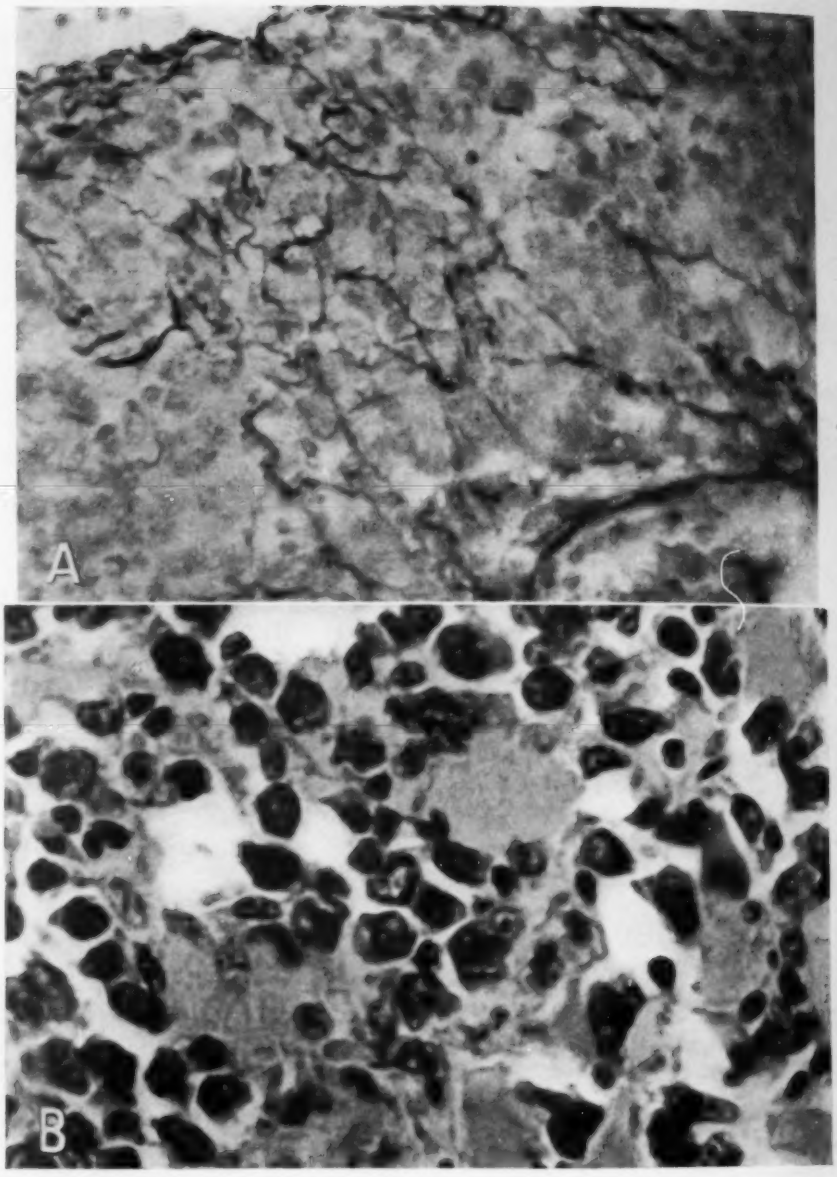

Fig. 2.—*A*, silver impregnation of reticulum (rat 108). Most of the reticulum radiates out from blood vessels. *B*, tumor cells among muscle fibers in the wall of a large pulmonary vein (rat 241).

Both the infectious processes and the lymphosarcoma were more frequent in right than in left lungs. There may be a relation between chronic infection and lymphosarcoma of the lung in rats, but in our material it was not definite; there are certainly other factors, especially that of age.

It is well understood that the presence of pulmonary infections and other spontaneous diseases in older rats renders imperative in any experiments on chronic toxicity such as those referred to in this paper the use of untreated groups of control animals for comparative purposes. The experimental substances used in this series (food and drug dyes and solvents in low concentrations, usually 0.01 per cent of the diet for the dyes and 1 and 2 per cent of the diet for the solvents) showed no tendency to accelerate or originate the lymphosarcoma. In the group of 135 rats over 2 years of age 35 were untreated controls; 10 of these had lymphosarcoma, a percentage of 28.6 as against 22.2 for the group as a whole and 20.0 for the treated animals only.

A relationship between infection and lymphosarcoma may have been present in the series studied by Bullock and Curtis. They noted that over half of their rats with lymphosarcoma of the mesenteric nodes showed macroscopic signs of cecal ulceration; our series was entirely free from cecal ulceration and in no rat was lymphosarcoma primary with any certainty in the mesenteric lymph nodes (in 3 this was possible but, we believe, not probable).

REVIEW OF THE LITERATURE

For a discussion of lymphosarcoma and of lymphoblastoma in general in mice, the reader is referred to an extensive paper by Simonds.[2] Among 15,000 mice he found 316 with enlarged lymph nodes; among these were 51 mice with lymphosarcoma, intrathoracic in the majority. Recent discussions of lymphosarcoma in man are those by Ewing and by Sugarbaker and Craver, cited in our comment, page 584.

Before 1930, the only report of a lymphosarcoma in a rat appears to have been that of Woolley and Wherry;[3] several reports of the condition in mice had appeared.[4] In 1930 Bullock and Curtis[5] reported lymphosarcoma of the mesenteric nodes in 78 and lymphosarcoma of the mediastinum, lung and thymus in about 15 of 489 rats with spontaneous tumors; metastases to the peritoneum and to other lymph nodes were frequent. McEuen[6] encountered among several thousand rats 99 with lymphosarcoma of the ileocecal nodes; metastases were observed frequently in mesenteric and thymic lymph nodes and occasionally in

2. Simonds, J. P.: J. Cancer Research **9**:329, 1925.
3. Woolley, P. G., and Wherry, W. B.: J. M. Research **25**:205, 1911.
4. Opie, E. L.: Medicine **7**:31, 1928.
5. Bullock, F. D., and Curtis, M. R.: J. Cancer Research **14**:1, 1930.
6. McEuen, C. S.: Am. J. Cancer **36**:383, 1939.

viscera. Jenney [7] recently reported the transplantation "with difficulty" of a reticulum cell lymphosarcoma of the rat through twelve generations; this tumor was found in 21 of 183 rats; it originated in the mesenteric lymph nodes and showed metastases to the spleen, liver, kidneys, mediastinum and lungs. Bullock and Curtis had grafted portions of one tumor into the subcutaneous tissues of 24 rats, with negative results.

COMMENT

Histologically, this tumor fits closely into the group of reticulum cell lymphosarcoma, following the recent classifications of Ewing [8] and Sugarbaker and Craver.[9] It corresponds to the older "large cell lymphosarcoma" and to the polymorphous reticulum cell sarcoma of Roulet [10] and Ahlström.[11] It resembles closely the polymorphous cell rat lymphosarcoma illustrated by Bullock and Curtis.[5] We should emphasize that there is great variation in the amount of argentophilic reticulum which the reticulum cell lymphosarcoma may produce.

A possible relation between lymphosarcoma and infection has been discussed.

The age which our rats attained before lymphosarcoma became frequent was 745 days, and the earliest appearance observed was at 467 days. In the Bullock and Curtis series of lymphosarcomatous rats the ages ranged from 278 to 734 days, with an average of 421 days.

In addition to the 35 tumors of this type found in the 323 rats under discussion in this paper, there were found also 25 spontaneous malignant and benign tumors, chiefly embryonal mixed tumors of the kidney, subcutaneous fibrosarcoma, mammary fibroadenoma and hepatic adenoma. Two mammary tumors diagnosed as fibroadenoma and a tumor of the liver diagnosed as adenoma occurred in animals also having lymphosarcoma.

SUMMARY

Few series of cases of lymphosarcoma in the rat have been reported. Among 323 rats we found 35 with pulmonary lymphosarcoma of the reticulum cell type, originating in the lymphoid accumulations around the large bronchi. The mediastinal lymph nodes and thymus were never the source of the tumor. More or less extension into the mediastinum was present in 23 of 35, and metastases in lymph nodes or viscera occurred in 6 of the 35 tumor-bearing rats. Thirty of the 35 rats were over 24 months of age.

7. Jenney, F. S.: Transplantation of a Reticulum Cell Lymphosarcoma of the Rat Through Twelve Generations, read at the Thirty-Third Annual Meeting of the American Association for Cancer Research, Pittsburgh, March 20, 1940.
8. Ewing, J.: Bull. New York Acad. Med. **15**:92, 1939.
9. Sugarbaker, E. D., and Craver, L. F.: J. A. M. A. **115**:17, 1940.
10. Roulet, F.: Virchows Arch. f. path. Anat. **277**:15, 1930.
11. Ahlström, C. G.: Acta path. et microbiol. Scandinav. **16**:401, 1939.

RELATION OF EXPERIMENTAL PULMONARY ARTERIAL HYPERTENSION TO ARTERIOSCLEROSIS

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The purpose of this study was to determine whether pulmonary arterial hypertension can be produced by physical obstruction in the peripheral parts of the arterial tree and to learn whether or not such hypertension leads to the development of intimal sclerosis in the pulmonary arteries. The results were not wholly satisfactory, but the record is made for its negative as well as its presumptive positive results.

The pathology of the pulmonary arteries of man was fully reviewed by Brenner [1] in 1935. He adhered to the view that secondary pulmonary arteriosclerosis is due to increased intra-arterial pressure but expressed doubt that primary sclerosis of smaller pulmonary arteries produces right ventricular hypertrophy. In the latter case, according to Brenner, the sclerosis and right ventricular hypertrophy are due to some common cause and are not related to each other. He failed to consider that the sclerosis which is apparently secondary to pulmonary arterial hypertension may be due to the condition which underlies the hypertension rather than to the hypertension itself. Brenner emphasized the great reserve of the pulmonary circulation and pointed out that 75 per cent of the cross-sectional area of the pulmonary arterial system must be obliterated before systemic arterial pressure falls. He attached little significance to vasomotor innervation.

Since the time of Brenner's review further studies have appeared. Among them may be mentioned (1) the demonstration by Daly, Foggie and Herb [2] that epinephrine brings about an increase in pulmonary arterial pressure in the perfused lung of the dog, (2) the establishment by Bennett and Smith [3] of the fact that it elevates the pulmonary arterial

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This investigation was aided by a grant from the Josiah Macy Jr. Foundation.

1. Brenner, O.: Arch. Int. Med. **56**:211, 457, 724, 926 and 1189, 1935.

2. Daly, I. deB.; Foggie, P., and Herb, C. O.: Quart. J. Exper. Physiol. **30**: 21, 1940.

3. Bennett, G. A., and Smith, F. J. C.: J. Exper. Med. **59**:181, 1934.

pressure in the rat and (3) the finding of Katz and Steinitz [4] that in experimental renal hypertension of the dog the pulmonary arterial pressure is not increased.

Experimental studies of prolonged hypertension in the pulmonary arteries are not numerous. Kirch [5] injected metallic mercury intravenously into 12 rabbits. He found that the globules lodged in the peripheral pulmonary circulation, without passing through, and produced sterile abscesses. He reported that hypertrophy of the right ventricle, following tonogenic dilatation, appeared at the end of thirty-six days and that the left ventricle participated at the end of forty-five days. His method of examining the heart, by cutting it into six sections, does not provide a satisfactory distinction between the right and the left ventricle. Of interest is this contradiction of his earlier view that the chambers of the human heart undergo hypertrophy independently of each other.

Bennett and Smith [3] reported the occurrence of sclerotic disease of the pulmonary arteries in rats following prolonged exposure to compressed air having a barometric pressure of 3,040 mm. of mercury and an oxygen tension of 635 mm. of mercury. They showed that after twenty-four to thirty-one days of exposure significant pulmonary hypertension appeared. After three days of exposure there was thickening of the arterial walls with perivascular edema, followed by progressive thickening, narrowing and hyalinization of the pulmonary arterioles. The lesions of the vessels were not attributed to the hypertension. They may have been due to oxygen poisoning, although the studies of Rehbock, Oldt and Dixon,[6] who failed to find such changes in association with high oxygen concentrations at normal barometric pressure, indicate that the lesions may have been induced by the high barometric pressure rather than by the increased oxygen tension.

The method of Kirch [5] is not applicable to the problem under discussion, since any disease of the pulmonary arteries might well be due to the irritant properties of the mercury. That of Bennett and Smith [3] is not applicable, for the arterial lesions occur before the hypertension appears.

METHOD

Dogs were selected as experimental animals because the size of the dog's heart permits a reasonably accurate differentiation between the weights of the two ventricles.

In the earlier stages of the experiments, lycopodium (Pennick) in 1.0 per cent suspension in 6.0 per cent acacia in 0.85 per cent sodium chloride solution was injected into the external vein of a leg, without anesthesia. (The acacia was dissolved in the saline solution in a water bath and then strained through four layers

4. Katz, L. N., and Steinitz, F. S.: Am. J. Physiol. **128**:433, 1940.

5. Kirch, E.: Centralbl. f. allg. Path. u. path. Anat. **58** (supp.):103, 1933.

6. Rehbock, D. J.; Oldt, M. R., and Dixon, H. M.: Arch. Path. **30**:1172, 1940.

of gauze. The lycopodium was added to small amounts of this solution and the mixture shaken by hand to obtain an even suspension. This was sterilized in the Arnold sterilizer, care being taken to prevent boiling over of the mixture, and then kept in sterile flasks at 4 C. Control injections made with 25 per cent sucrose in 12 per cent acacia produced no reaction.)

Later it was decided to use a larger type of seed, and the seeds of the vanilla bean were selected. These have roughly a diameter of about 400 microns. The vanilla bean is opened lengthwise and the pulp scraped out. The mass is then boiled in 95 per cent ethyl alcohol, with reflux condenser, and the vanillin extracted. The mass now consists of seeds and fibrous material, the largest particles of which are picked out. The mass is then boiled in 1 per cent sodium hydroxide solution for three minutes, the solids being added after the solution has come to a boil. The hydrated extraneous material floats to the top and is removed. Repeated washing in cold water separates all the other material from the seeds.

The water is removed from the seeds by boiling in 95 per cent alcohol with reflux condenser. The seeds are removed on filter paper and then are boiled for six hours in 100 per cent ethyl alcohol. They are again removed on filter paper and are boiled in ether to remove the alcohol and resinous material. After six hours' boiling, the old ether is replaced with new and the mixture boiled for another six hours. The seeds are removed on filter paper, dried, weighed in small quantities and autoclaved.

Twenty-four to forty-eight hours before use, the seeds are mixed in 0.85 per cent sodium chloride solution containing 10 per cent acacia and 20 per cent sucrose, a solution the specific gravity of which approximates that of the seeds. One drop of caprylic alcohol is added to prevent foaming and the mixture placed in an electric shaker for from two to three hours. The suspension lasts well for several minutes and can be restored by a few minutes' shaking by hand. This material was injected repeatedly into the external vein of a leg.

Only 10 animals survived long enough to be regarded as satisfactory. Thirty-nine others were used for preliminary study and control. Among these were 11 which were placed in the experiment but died from infection or embolism before they had been observed long enough for use in the series.

At the beginning of the study, roentgenograms were made before and after injections, but the differences in transverse measurement of the heart shadow were so slight and inconstant that this method was abandoned. Studies of corpuscular volume gave no consistent differences betwen controls and experimental animals, and were not continued. The studies of pulmonary arterial pressure will be given. Some of the animals were placed on the electric treadmill until hyperpnea developed, and this was repeated daily over the course of several months. Others treated similarly otherwise were not exercised and served as controls for this exercise.

Table 1 gives the essential data of the experiments with lycopodium and with vanilla bean seeds. As a result of the experiments with the spores it became evident that lycopodium is not effective in producing any change in weight of the right ventricle indicative of pulmonary arterial hypertension. It was not deemed necessary to use new animals, so those "seasoned" to the spores were treated with vanilla bean seeds. In the table it is shown that each period of injections followed a period without injections. Those animals exercised were placed on the treadmill daily to the end of the experiment regardless of injections. The weight of each animal is given as at the time of the beginning of the experiments.

TABLE 1.—*Data on Injections*

Dog, Weight, Sex	Lycopodium			Vanilla Bean Seeds			Exercise, Mo.	Duration of Experiment, Mo.	Fate of Animal
	Inclusive Dates	Injections	Total Volume of Suspension, Cc.	Inclusive Dates	Injections	Total Volume of Suspension, Cc.			
60 10.6 Kg. F	12/22/32- 6/1/33	12	112	10/30/34- 2/6/35	10	100	19	63	Put to death
				5/27/35- 10/16/36	9	255			
				12/14/36- 1/1/38	25	507			
75 12 Kg. F	12/22/32- 6/1/33	13	127	10/30/34- 2/6/35	11	110	None	30	Died after last injection—85 cc.
				5/27/35- 5/31/35	3	195			
80 11 Kg. M	12/22/32- 6/1/33	13	120.5	10/30/34- 2/6/35	10	100	None	30	Died after last injection—about 50 cc.
				5/27/35- 5/31/35	3	180			
90 7.6 Kg. F	1/5/33- 6/1/33	11	110	10/30/34- 2/6/35	10	100	None	26	Killed in dog fight
92 8.6 Kg. F	1/26/33- 4/27/33	10	87	10/30/35- 10/16/36	19	315	3½	48	Died after exercise
				12/2/36- 1/4/37	11	232			
94 14.1 Kg. M	1/26/33- 6/1/33	12	112	10/30/34- 2/6/35	10	100	8	52	Killed in dog fight
				5/27/35- 10/16/35	9	290			
				12/14/36- 5/20/37	21				
96 F	1/26/33- 6/1/33	10	97.5	10/30/34- 2/6/35	10	100	17	61	Put to death
				5/27/35- 10/16/35	11	240			
				12/12/36- 6/16/37	22	472			
97 10.1 Kg. F	1/26/33- 6/1/33	12	109	10/30/34- 10/16/35	19	265	None	63	Put to death
				12/14/36- 6/6/37	25	547			
106 8.6 Kg. F	1/26/33- 6/1/33	12	103	10/30/34- 10/16/35	19	350	9	53	Died after last injection—25 cc.
				12/14/36- 6/16/37	25	575			
				9/29/34- 11/23/34	5	50			
S 11.5 Kg. F	.....	..	..	5/27/35- 10/16/35	9	235	None	27	Died after last injection—15 cc.
				12/16/36- 1/4/37	10	165			

With the aid of Paul Gebauer, of the department of surgery, attempts were made to prepare dogs for direct measurement of pressure in the pulmonary artery. The first method was that of bringing the left pulmonary artery up to the surface and anchoring it under the skin after local thoracoplasty. Twelve animals were operated on either in one or two stages. This method was abandoned because the technical difficulties led to death by infection or hemorrhage. Even when the animal survived for several days the difficulty in locating the "exteriorized" artery made readings undependable. The Daly method of inserting into the thorax a flanged aluminum cannula in contact with the pulmonary artery, so that a needle could be inserted into the artery for pressure determinations, was also tried. Of 7 animals, the experiment was successful in 1. On two days the pressure ranged from 19 to 30 mm. of mercury and then the artery became occluded by a thrombus. The method, in our hands, proved unsuitable for prolonged experiments.

The experiments on dogs 60, 96 and 97 were terminated by measurements of the pulmonic arterial pressure by D. E. Gregg, of the department of physiology. Measurements were made with closed and open thorax. The fact that the animals were not trained for this purpose left some doubt as to the accuracy of the readings. Neither in dogs 60 and 96, which had been exercised on the treadmill, nor in dog 97, which had not been so exercised, were the readings above those of trained controls, although all were near the upper limits of normal controls. Thus no significant increase of pulmonic arterial pressure was demonstrated in these animals.

It might be assumed, however, that if an elevation of slight degree were maintained over a period of many months there would be cardiac hypertrophy. Whereas in such animals as the rat and the rabbit the demonstration of alteration in the ratio of heart weight to body weight is not difficult, the matter is different in the dog. Control observations show such wide variation that even dogs with persistent systemic hypertension of high degree show only trends toward hypertrophy of the heart. For example, the ratio in 50 control dogs varied from 3.8 to 12.8 but there were only 2 below a ratio of 6 and the mode lay in the range of 6 and 7.

The ratio of the weights of the left and right ventricles is also variable in the dog. Herrmann,[7] whose method was used in this study, found the average to be 1.39, but the minimum was 1.15 and the maximum 1.77. When the method was checked on 5 control dogs, the ratio varied from 1.31 to 1.76.

Positive identification of hypertrophy by microscopic examination can be assured only by large scale measurements of cross sections of fibers and also of nuclei. In the dog the attenuation of fibers by dilatation introduces a disturbing feature. The same is true concerning nuclei, but to a less degree. Many of the animals died immediately after receiving an injection or after exercise, and the right side of the heart was markedly dilated. When the rounded ends of the nuclei become squared, hypertrophy is usually thought to be present.

Table 2 gives the data on the heart weight–body weight ratio, the left-right ventricle weight ratio and the transverse measurement of the nuclei in both ventricles. The ratio of heart weight to body weight gives little information except that in regard to dogs 90, 96 and 106 it might be thought that the trend is toward hypertrophy. The ratio of the weights of the left and right ventricles shows a significantly low figure only in dog 106, but the trend may be thought to be present in dogs 75 and 92. The average transverse diameter of the nuclei is increased in one or both ventricles in all the animals. The controls show transverse measurements of 6.5 to 7.9 microns. In all the hearts, the nuclei showed squared ends.

7. Herrmann, G. R.: Am. Heart J. **1**:213, 1926.

Complete autopsies were performed on all the animals. Right ventricular dilatation was a frequent observation, especially notable in dog 75. The lungs showed some degree of emphysema in all cases. The seeds were massed in small arteries and arterioles. They had excited a low grade exudative inflammation in those animals which died or were killed in short experiments. In the 10 experimental animals reported on in detail, the masses were infiltrated with and surrounded by dense collagenous connective tissue, resembling a healed granulomatous lesion.

Not infrequently the small arteries immediately proximal to the seeds and their capsule showed slight fibrosis of the intima. Careful examination of the main stem of the pulmonary artery and its larger branches failed to demonstrate intimal fibrosis or medial hypertrophy.

TABLE 2.—*Heart Weight Ratios*

	Weight (Gm.)* of				Ratio Heart Weight to Body Weight	Ratio of Right to Left Ventricle Weight	Cross Section of Nuclei, Microns †	
Dog	Dog	Heart	Left Ventricle	Right Ventricle			Left Ventricle	Right Ventricle
60	15,000	94.2	40.4	31.8	6.28	1.27	8.9	8.8
75	26,400	....	65.6	55.1	....	1.19	8.2	6.5
80	15,000	....	56.3	42.6	....	1.32	7.5	8.6
90	10,400	95.0	38.5	30.0	9.13	1.28	9.3	8.0
92	10,500	82.4	34.9	31.6	7.85	1.10	8.9	9.3
94	16,900	135.0	76.6	52.7	7.99	1.46	9.2	8.4
96	15,500	155.0	68.4	41.2	10.00	1.66	8.3	8.2
97	17,000	125.0	52.8	34.4	7.35	1.53	8.08	8.7
106	10,900	100.0	40.1	44.0	10.00	0.91	8.5	9.1
8	11,500	92.5	37.8	29.6	8.04	1.27	8.2	8.3

* The weight of the heart is given as the weight in the fresh state. The weights of the separate ventricles represent constant weights after fixation in 4 per cent solution of formaldehyde. Fresh weights were not recorded for dogs 75 and 80. The weight of each animal is that at the time of death.

† The transverse diameter of the nuclei is an average of the measurements of not less than 100 to 180 nuclei.

SUMMARY

The lodgment of large numbers of lycopodium spores and vanilla bean seeds in the smaller pulmonary arteries and arterioles of dogs led to low grade exudative inflammation, followed by cicatrization and encapsulation. Conclusive evidence that pulmonary hypertension was produced was not found by physiologic methods even though in the cases studied the pressures were in the region of the upper limits of normal. Determinations of the heart weight–body weight ratio showed at best only a slight trend toward increase in the weight of the heart. The configuration of the nuclei of the cardiac muscle and their average transverse diameter indicated that cardiac hypertrophy was induced, but with consideration of the probable factors of error it cannot be said that there was a difference between the hypertrophy of the left and that of the right ventricle. A few determinations of pressure within the left ventricle were made and failed to disclose systemic hypertension. Thus, such hypertrophy as existed was probably due to elevations of pressure within

the pulmonary artery, certainly of small degree but persistent over many months. Under these conditions the pulmonary artery in its main stem and larger branches showed no arteriosclerosis either grossly or microscopically.

CONCLUSIONS

What is probably a slight degree of pulmonary arterial hypertension, lasting over many months, is induced by the lodgment of large numbers of small seeds in the small arteries and arterioles of the lungs of the dog. The evidence of such hypertension is found in the squaring of the ends and the increase of the transverse diameters of the nuclei of the cardiac muscle. The probable slight increase of pressure, persistent and prolonged, does not cause pulmonary arteriosclerosis.

CHANGES IN THE URINARY TRACT AND OTHER ORGANS AFTER ADMINISTRATION OF THREE SULFANILAMIDE DERIVATIVES

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The ever extending therapeutic application of sulfanilamide derivatives makes it necessary to devote special attention to the possibility that organic lesions may be caused by the administration of these compounds.

Damage due to chronic application of sulfanilamide or some of its derivatives in human beings and in experimental animals has been studied and reported repeatedly in the literature. However, no comparative data are available on acute changes which may follow a single injection of a high dose of some of the derivatives.

The present communication deals in its main part with the macroscopic and microscopic appearance of certain organic changes seen in albino rats after a single intraperitoneal injection of the sodium salt of sulfapyridine (2-[paraaminobenzenesulfonamido]-pyridine), of sulfathiazole (2-[paraaminobenzenesulfonamido]-thiazole) and of sulfamethylthiazole (2-[paraaminobenzenesulfonamido]-4-methylthiazole). Where it is deemed necessary for the completeness and understanding of the pathologic picture a brief description of lesions occurring after repeated injections of the compounds is added.

MATERIAL AND METHOD

The greatest part of the animal material of this investigation originated from albino rats used in a study of the toxicity of the sodium salts of sulfapyridine, sulfathiazole and sulfamethylthiazole, the results of which have been reported in detail elsewhere.[1] Complete autopsies were made on more than 150 animals, and

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This investigation was aided by grants from the Sidney C. Keller Research Fund and Mr. Samuel R. Cohn.

1. (*a*) Lehr, D.; Antopol, W.; Churg, J., and Sprinz, H.: Proc. Soc. Exper. Biol. & Med. **45**:15, 1940. (*b*) Lehr, D.; Churg, J., and Antopol, W.: ibid. **45**: 447, 1940.

special attention was given to the appearance of the urinary tract. All organs were fixed in 20 per cent solution of formaldehyde U. S. P. for histologic examination. Pieces of liver were also fixed in alcohol for Best's carmine stain for glycogen. In some cases von Kossa's stain for calcium was used on kidney and liver sections.

PATHOLOGIC CHANGES OBSERVED

Acute lesions will be described as they appeared after the administration of the absolute lethal dose of each of the three compounds (sodium sulfapyridine, 1.5 Gm. per kilogram; sodium sulfathiazole, 1.22 Gm. per kilogram; sodium sulfamethylthiazole, 0.77 Gm. per kilogram). With these doses, death occurred within three hours after the injection of sulfapyridine (average for 10 animals), within eleven hours after the injection of sulfathiazole (average for 20 animals) and within twenty hours after the injection of sulfamethylthiazole (average for 20 animals).

There were marked differences in the appearance of the urinary tract in different animals and in different experiments. After injection of a single dose of sodium sulfathiazole the peculiar observation was made that large amounts of precipitate of the free compound were found in the renal papillae, ureters and bladder in every case (fig. 1 *A, B, C* and *D*), even if the animals died as early as two to three hours after the injection. This finding was investigated further with injections of sublethal doses and reported elsewhere in detail.[2] The precipitate in the renal collecting tubules occurs in a noncrystalline form, as demonstrated by examination of both unfixed and formaldehyde-fixed frozen sections of the papilla under polarized light. It has the form of small disks and globules approximately the size of a leukocyte (fig. 1 *D* and fig. 2 *A* and *B*). Analysis of the precipitate showed it to consist almost entirely of *free* sulfathiazole, in contrast with the high amounts of acetylated derivatives found in uroliths after chronic administration of sulfapyridine and sulfathiazole.[3] Since sulfathiazole, the most soluble of the three compounds, is the only one to give this phenomenon of massive acute precipitation in the urinary tract,[4] it was assumed that the high rate of absorption and of renal excretion and possible lack of reabsorption from the tubules may be responsible for the high concentrations in the urine, with resulting precipitation. The

2. Lehr, D.; Antopol, W., and Churg, J.: Science **92**:434, 1940.

3. (*a*) Antopol, W., and Robinson, H.: Proc. Soc. Exper. Biol. & Med. **40**: 428, 1939. (*b*) Gross, P.; Cooper, F. B., and Lewis, M.: ibid. **40**:448, 1939. (*c*) Molitor, H., and Robinson, H.: Arch. internat. de pharmacodyn. et de thérap. **62**: 281, 1939. (*d*) Rake, G.: van Dyke, H. B., and Corwin, W. C.: Am. J. M. Sc. **200**:353, 1940.

4. In recent experiments with a new sulfanilamide derivative, 2-(paraaminobenzenesulfonamido)-pyrimidine (sulfadiazine), it was found that a similar picture of massive acute precipitation in the tubules of the renal papilla occurs after administration of this compound.

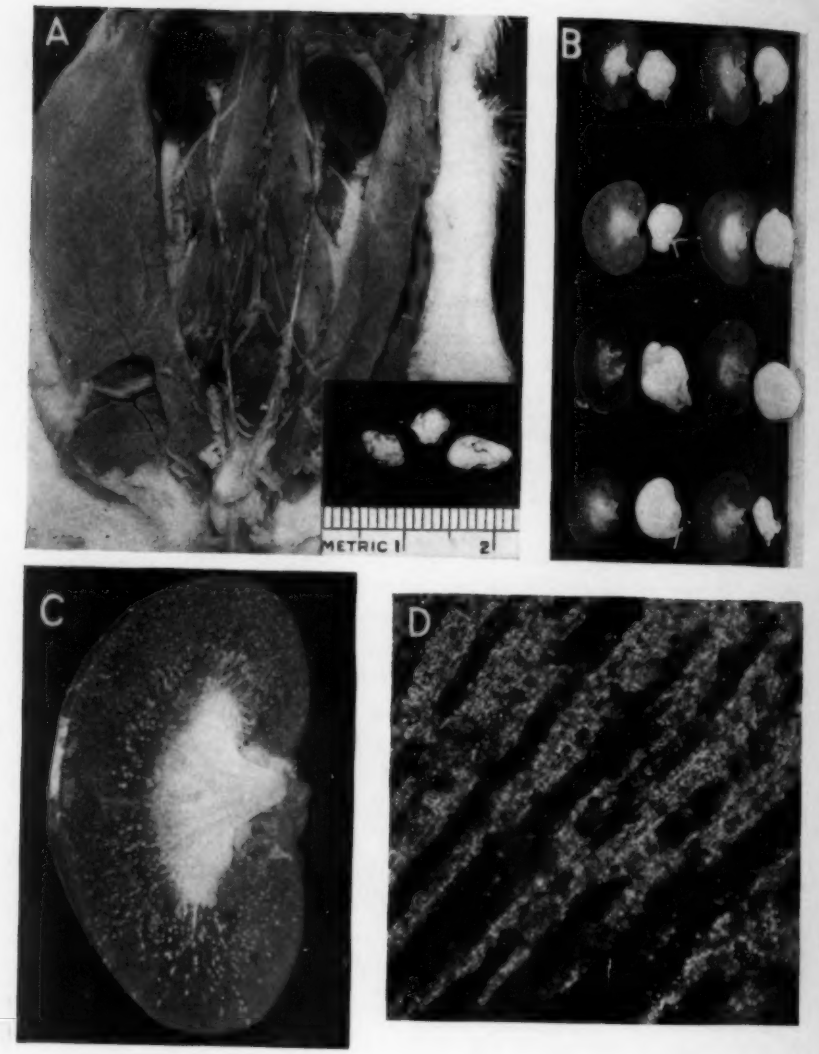

Figure 1

(See legend on opposite page)

same view, derived from clinical observations, was expressed by Reinhold, Flippin and Schwartz [4a] and by Pepper and Horack.[5] If a rat survived a single high dose of sulfathiazole, the precipitate was usually washed out of the kidneys within twenty-four hours. Occasionally, however, it was found obstructing the renal papilla even two days or more after the injection. Analysis of the precipitate in the latter case revealed the presence of considerable amounts of the extremely insoluble acetylated compound, which was probably responsible for the difficulty in redissolving the precipitate.

Signs of irritation, such as edema and congestion, were seen in the kidneys but passed off quickly if the precipitate was rapidly washed out, so that the obstruction lasted only for several hours. However, if the precipitate remained in the renal papilla for twenty-four hours or more, the lesions became much more pronounced. The kidneys in the latter case appeared very large and soft. They were pale yellowish green and showed on cross section a pale cortical region in contrast with a deeply congested medulla. On histologic examination there was, aside from the dilatation of tubules and glomerular spaces and the formation of globoid bodies, severe parenchymatous degeneration of distal convoluted tubules and collecting tubules, with considerable calcification of both of the latter structures (fig. 3 *C*).

This picture of calcifying nephrosis was seen occasionally after only a single injection of sulfathiazole, but it could be easily produced in a high percentage of animals by repeated daily injections of even

4a. Reinhold, J. G.; Flippin, H. F., and Schwartz, L.: Am. J. M. Sc. **199**:393, 1940.

5. Pepper, D. S., and Horack, H. M.: Am. J. M. Sc. **199**:674, 1940.

EXPLANATION OF FIGURE 1

Fig. 1.—Acute precipitation of free sulfathiazole in the urinary tract. *A*, urinary tract of a rat that died one hour after an intraperitoneal injection of sodium sulfathiazole in the dosage of 1.25 Gm. per kilogram. Note the white precipitate (sulfathiazole) in both ureters and bladder. The inset shows concrements from the bladder, consisting of free sulfathiazole. These were formed from the loose precipitate within twenty-four hours.

B, cross sections of kidneys and the bladders of rats that were killed or that died twenty, forty-five, ninety and one hundred and fifty minutes (respectively from top to bottom) after the intraperitoneal injection of sodium sulfathiazole in the dosage of 1.25 Gm. per kilogram. Note that the precipitate in the renal papilla is at the maximum after twenty minutes (top row) and that the bladders become progressively filled with the precipitate.

C, section of kidney from top row of *B* enlarged.

D, microscopic picture of the precipitate in the collecting tubules in unfixed and unstained frozen section. Note the appearance of the precipitate as disks and especially as globules of varying size, best visible in the right lower corner of the photograph (× 105).

much smaller doses (e. g., three to five intraperitoneal injections of 0.5 Gm. per kilogram of sodium sulfathiazole). It was interesting to note that the right and left kidneys could be affected differently. For instance, in one case the left kidney showed a narrow white zone (calcification of collecting tubules) separating the renal medulla from the cortex (fig. 3 *D*) and no precipitate in the papilla, while the right kidney was completely obstructed by precipitate and showed more dif-

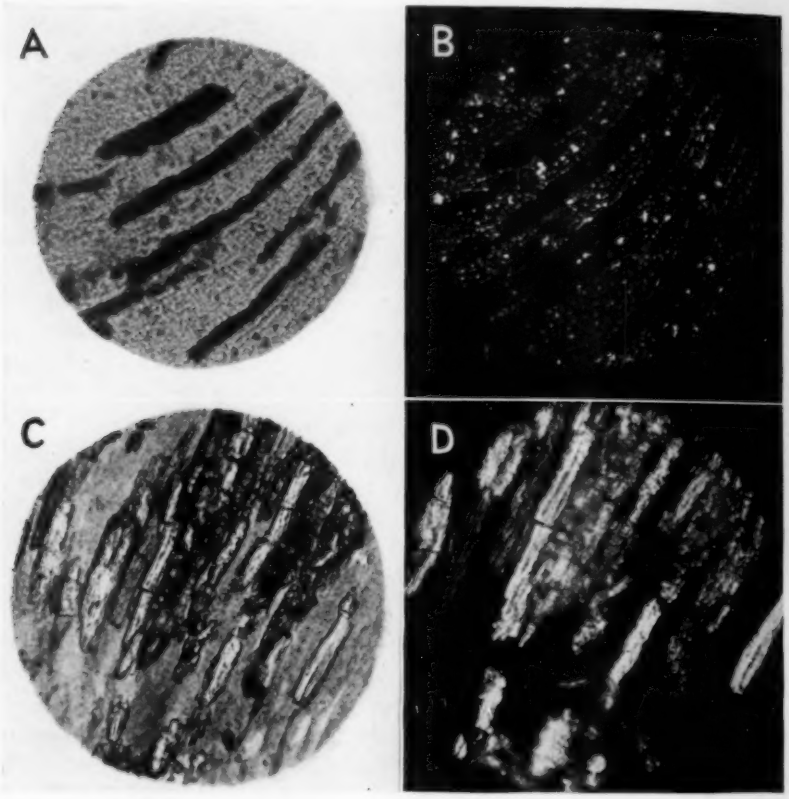

Fig. 2.—Appearance of drug precipitate in renal collecting tubules under normal and under polarized light. *A* and *B* show an area containing a precipitate of "free" sulfathiazole (× 96): *A*, under normal light; *B*, under polarized light (Nicol prisms crossed). Note that the areas containing the precipitate appear completely blacked out, while crystals of sulfathiazole added to the fixative appear as white dots. *C* and *D* show an area containing a precipitate of acetylsulfapyridine (× 96): *C*, under normal light (note the crystalline structure); *D*, under polarized light. The precipitate shows double refraction.

fuse calcification in the cortex. A difference in lesions of the right and left kidneys was also often found after administration of sulfapyridine [3a] and sulfamethylthiazole.

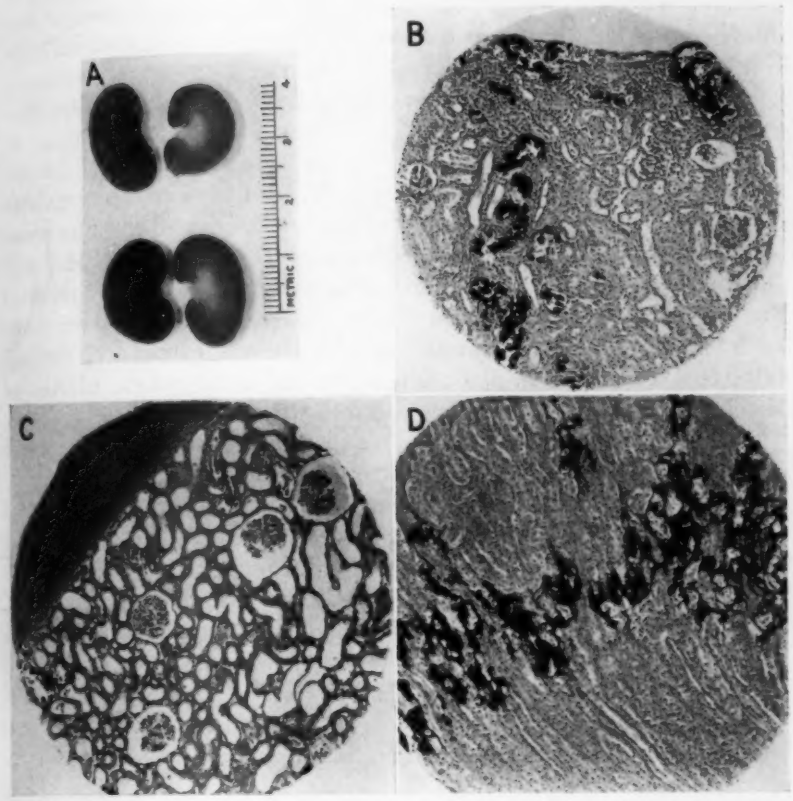

Fig. 3.—Calcifying nephrosis after injection of sulfathiazole and of acetylated sulfapyridine. *A*, gross appearance of kidneys of rats killed four days after a single intravenous injection of sodium acetylsulfapyridine in the dosage of 0.75 Gm. per kilogram. The surface of the organ shows whitish, somewhat elevated dots, which on cross section extend as white lines into the cortical region.

B, microscopic appearance of renal cortex from a kidney in *A*. Note the calcification of distal convoluted tubules (von Kossa calcium stain; × 56).

C, renal cortex of a rat after a single intraperitoneal injection of sodium sulfathiazole in the dosage of 0.75 Gm. per kilogram. (The animal was killed four days after the injection and showed the papillae obstructed by large amounts of precipitate.) Note the dilatation of the glomerular spaces and tubules. The latter show parenchymatous degeneration and areas of calcification. The left upper corner shows a perirenal hemorrhage (hematoxylin-eosin stain; × 56).

D, zone of calcification (in the collecting tubules and peritubular zones) separating the renal cortical from the medullary region, after repeated injections of sulfathiazole (von Kossa calcium stain; × 72). (The rat received two intraperitoneal injections of sodium sulfathiazole on two consecutive days in the dosage of 0.5 Gm. per kilogram and died about forty-eight hours later.)

A strikingly high incidence of precipitation in the renal papillae was seen even after comparatively small doses (0.5 Gm. per kilogram) of sodium sulfamethylthiazole. The quantity of the precipitate, however, was much smaller and formed at a slower rate than in the case of sulfathiazole, appearing only after about twelve hours. The sulfamethylthiazole concretions were situated partly in the papillae but chiefly in the renal pelves, ureters and bladder. The extrarenal precipitate consisted of many single concrements. (Analysis of the stones as well as of the precipitate in the papillae showed them to be about 30 per cent acetylated derivative.) The observations described are in agreement with those of Gross, Cooper and Scott,[6] who studied results of chronic oral administration of sulfathiazole and sulfamethylthiazole. They reported that concretions in renal tubules were encountered almost three times as frequently with sulfathiazole as with sulfamethylthiazole, whereas extrarenal concrements, i. e., stones in the renal pelves, ureters or bladder, were twice as common with the latter drug.

A significant observation in our experiments was the fact that the presence of sulfamethylthiazole concrements in the kidneys was invariably accompanied by severe degeneration of the liver as well as of the kidneys. The kidneys appeared enlarged, pale greenish yellow, very soft and edematous. Serosanguinous fluid dripped from the fresh cross section, and the kidney tissue "lipped" over the capsule. Precipitation, usually extending over the entire papilla, was seen filling the lumens of the papillary ducts (fig. 4 *A*). The ureters were dilated, and blood-tinged urine was found in the bladder. In addition, many animals showed concrements in the renal pelves, ureters and bladder. Histologic sections in many cases revealed marked dilatation of the glomerular spaces, collecting tubules and papillary ducts. The outline of the epithelial cells in many distal convoluted tubules was indistinct, and the nuclei were very pale (fig. 4 *B*). The tubular lumens contained numerous globoid bodies and occasionally basophilic coagulum. Rats which survived a large dose and were put to death three to six days after the injection showed, in addition, areas of calcification in the distal convoluted tubules and sometimes also in the collecting tubules.

The liver was enlarged, yellowish brown, soft and very friable. The edge was rounded. The cut surface showed a conspicuous lobular pattern (red dots in a yellowish network). On microscopic examination the picture was that of severe degeneration. Sections from the liver, fixed in absolute alcohol immediately after death and stained with Best carmine, were devoid of glycogen. This finding was confirmed by chemical analysis.

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6. Gross, P.; Cooper, F. B., and Scott, R. E.: Urol. & Cutan. Rev. **44**:205, 1940.

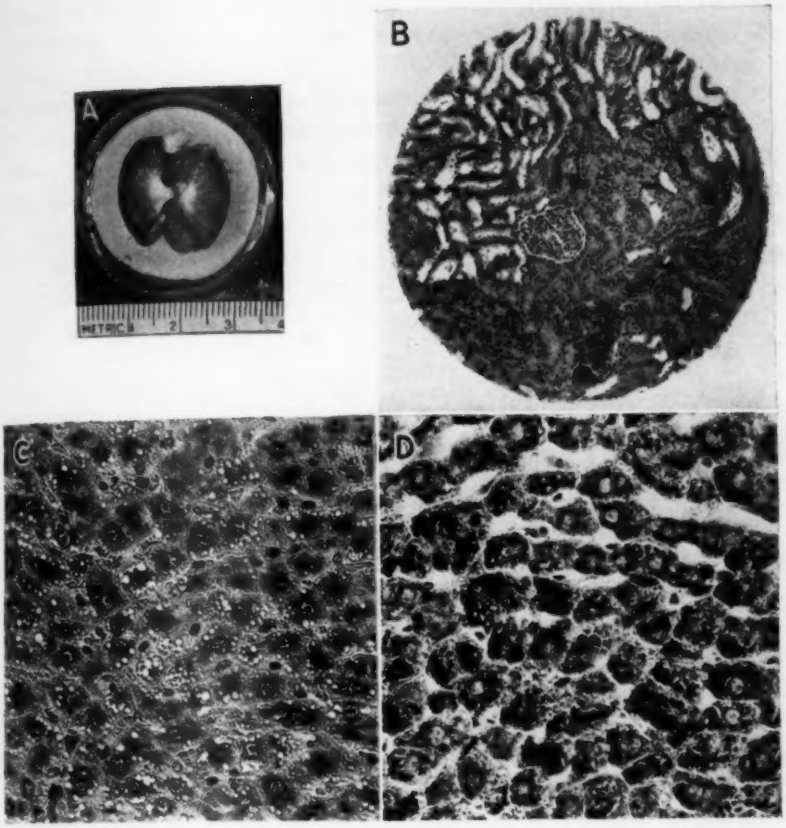

Fig. 4.—Degeneration of kidney and liver after administration of sulfamethylthiazole. *A*, typical gross appearance of adrenal and kidney. Both organs are considerably enlarged, with drug precipitation in the renal papilla. (The rat was killed one day after four intraperitoneal injections of sodium sulfamethylthiazole on four consecutive days in the dosage of 0.5 Gm. per kilogram.)

B, microscopic appearance of kidney. Parenchymatous and fatty degeneration of tubules are shown, with globoid bodies and hemorrhages, in the renal cortex (hematoxylin-eosin stain; × 56). (The rat died twenty-six hours after an intraperitoneal injection of sodium sulfamethylthiazole in the dosage of 0.75 Gm. per kilogram.)

C, the appearance of the liver cells in the periportal zones after repeated injections of sulfamethylthiazole (hematoxylin-eosin stain; × 290). Note vacuolation. (The rat was killed two days after three intraperitoneal injections of sodium sulfamethylthiazole on three successive days in the dosage of 0.5 Gm. per kilogram.)

D, the appearance of liver cells after repeated injections of sulfapyridine. Note the large amount of glycogen and the absence of vacuolation (Best carmine stain; × 290). (The rat was killed after eight intraperitoneal injections of sodium sulfapyridine within sixteen days, the total dose being 6.5 Gm. per kilogram.)

After repeated injections the liver cells at the periportal fields of the lobules revealed, in addition, distinct vacuolation (fig. 4 *C*). Sudan IV stain on frozen sections showed these cells to contain considerable amounts of fat.

An explanation for these parenchymatous lesions was found in studies of renal function and the concentrations of these drugs, in the blood and liver, which revealed that precipitation of acetylated sulfamethylthiazole in the collecting tubules of the kidneys caused pronounced impairment of the excretory function with accumulation of toxic amounts of the drug in the blood and tissues. Since impairment of renal function in itself, even if severe (e. g., that induced by intravenous injection of sodium acetyl-sulfapyridine), does not cause any of the aforementioned lesions in the liver, the degeneration of the parenchymatous organs is believed to be due mainly to the high concentrations of sulfamethylthiazole per se. Detailed data were reported elsewhere.[1b]

The livers of rats receiving sulfathiazole or sulfapyridine were enlarged, grayish red and had rounded edges. In histologic sections, the liver cells were enlarged, and the cytoplasm contained both coarse and fine basophilic granules. Alcohol-fixed tissue stained with Best carmine showed large amounts of glycogen (fig. 4 *D*). The same picture has been reported in a previous communication [7] as observed after chronic oral administration of sulfapyridine.

Renal concrements were not found after a single injection of the same amounts of sulfapyridine, and as a rule neither a single dose of sulfapyridine nor one of sulfathiazole produced the severe renal lesions that were usually found after a single dose of sulfamethylthiazole.

In the case of sodium sulfapyridine, the absence of precipitation in the urinary tract can be explained by the early death resulting from the administration of large amounts of the drug. Thus, there was insufficient time for the formation of the very insoluble acetylated compound. In addition, extensive concentration of the free compound in the urine does not occur, because of the comparatively slow absorption and excretion of the compound and the probable reabsorption of free sulfapyridine from the renal tubules.[8]

Sulfathiazole, however, which is more than twice as soluble as sulfapyridine, is absorbed and excreted rapidly and apparently not reabsorbed from the tubules to any considerable extent. Consequently, though only small amounts will be converted into the insoluble combined form, the quantity of free compound in the urine can easily exceed the amount that would be soluble. The copious precipitate of

7. Antopol, W., and Robinson, H.: Arch. Path. **29**:67, 1940.

8. Taylor, L. F. H.; Lowell, F. C.; Adams, M. A.; Spring, W. C., Jr., and Finland, M.: J. Clin. Investigation **19**:201, 1940.

the relatively soluble free sulfathiazole which forms in the collecting tubules is constantly being washed down, accumulating in the ureters and bladder, where it finally forms hard aggregates (within twenty-four hours; see inset in fig. 1 *A*). After the blood is cleared of the drug, the intrarenal precipitate is completely eliminated from the kidney by the oncoming urine. Sulfathiazole, therefore, does not usually remain in the normal kidney for a sufficient length of time to cause serious damage.

In contrast with sulfathiazole, sulfamethylthiazole is even less soluble than sulfapyridine. The slow excretion enables considerable acetylation to occur, with the accumulation in the kidneys of appreciable amounts of extremely insoluble acetylated derivative. The precipitate, therefore, even if comparatively sparse, will remain in the kidneys and cause severe irritation of the tubular epithelium, with all of the consequences described.

It seems worth mentioning that after administration of each of the three compounds, the adrenals frequently were enlarged and congested. On histologic examination the cells of the zona fasciculata showed deeply eosinophilic, almost homogeneous cytoplasm with an occasional vacuole.[7] At times minute foci of necrosis were present in the medulla. Pronounced vacuolation was found after repeated injections of sulfamethylthiazole. In a few instances the ganglion cells of the medulla also contained vacuoles.

After chronic administration of each of the three compounds there is ample opportunity for the formation of insoluble acetylated derivatives. Therefore concrements in the urinary tract will be frequent. As the appearance in the urinary tract of precipitates containing acetylated compounds was often accompanied by calcifying nephrosis, the mechanism of this severe and permanent damage to the kidney was investigated by injecting intravenously acetylsulfapyridine and acetylsulfathiazole in the form of their sodium salts.

This investigation revealed that a single intravenous injection of a high dose of the acetylated derivative which causes massive precipitation of the compound in the renal papilla, in rats as well as in mice,[9] is invariably followed by extreme degeneration of tubules, with ensuing calcification. The calcifying nephrosis involves mainly the distal convoluted tubules and remains visible for many weeks after the administration of the drug. This picture has been reported briefly [10] and will

9. Molitor, H., and Robinson, H.: Proc. Soc. Exper. Biol. & Med. **41**:409, 1939.

10. Antopol, W.: Arch. Path. **30**:385, 1940.

be followed by a detailed description in the near future. Frequently pronounced calcification of the media of the arterial tree, especially of that of the aorta, was found accompanying the renal lesion desecribed.

In conclusion it can be said that greatest caution is advisable in the use of sulfamethylthiazole, since this drug is apt to cause severe lesions in both kidneys and liver. As regards the other two compounds, sulfathiazole seems to be more apt to produce renal damage than sulfapyridine, because of the higher insolubility of its acetylated compound and the possibility of acute precipitation of free sulfathiazole in the urinary tract. In clinical cases, of course, the lesions of the kidneys may be expected to be much less severe and their incidence much lower than in animal experiments, in which the doses are in general many times those used in human beings.

SUMMARY

The macroscopic and microscopic pictures of renal damage after single and repeated intraperitoneal injections of the sodium salts of sulfapyridine, sulfathiazole and sulfamethylthiazole in albino rats are described.

A high incidence of intrarenal precipitation and urolith formation, accompanied by severe damage to the kidneys and liver, was seen after the injection of sodium sulfamethylthiazole.

The administration of high doses of sodium sulfathiazole was invariably followed by acute massive precipitation of the free drug in the urinary tract. This phenomenon is assumed to be due to the high rate of elimination and the lack of reabsorption of this compound from the renal tubules. The precipitation in the collecting tubules was found to occur in a noncrystalline form.

With chronic administration, renal concrements could be produced with each of the three compounds and were due to the formation of the very insoluble acetylated derivatives of these compounds. The appearance of the concretions was usually accompanied by calcifying nephrosis of varying degrees, mainly of the distal convoluted tubules, sometimes also of the collecting tubules. A single intravenous injection of any of the acetylated derivatives in the form of the sodium salt produced severe, extensive calcifying nephrosis of the type described.

BRAIN DEGENERATION IN YOUNG CHICKS REARED ON AN IRON-TREATED VITAMIN E–DEFICIENT RATION

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In an extensive series of feeding experiments in which young chicks were maintained on a synthetic ration deficient in vitamin E, Pappenheimer and Goettsch [1] studied a condition of brain degeneration which they designated "nutritional encephalomalacia." The condition was originally attributed to the lack of vitamin E in the ration—a conclusion which was modified after it was found that wheat germ oil would not entirely prevent the condition. Their latest work,[1b] however, and the investigations of other workers, notably Dam and co-workers,[2] lend support to their original view that vitamin E deficiency is involved. Experiments which I have carried on have produced a similar condition of the brain in feeding tests in which vitamin E deficiency is involved, but there are indications that some other factor is also implicated.

These studies were begun about the same time that Pappenheimer and Goettsch began their work and have consisted of feeding trials with young chicks in which a ration was used which had been treated with ferric chloride for the purpose of destroying vitamin E. It is noteworthy that Pappenheimer and Goettsch never obtained the reaction with iron-treated food, but the methods employed in the present experiments, as will be pointed out, entailed a slight modification of the original Waddell-Steenbock process,[3] which probably accounts for the results. In the earliest of these experiments there suddenly developed in a number of chicks, during the third or fourth week after hatching, a condition of imbalance, and at autopsy the brains showed hemorrhage and disorganization (fig. 1 *B*). Since that time, during repetitions of these feeding trials the condition has appeared in many lots, usually with considerable regularity as to the time of occurrence, but always some-

From the Department of Zoology, University of Illinois.

1. (*a*) Pappenheimer, A. M., and Goettsch, M.: J. Exper. Med. **53**:11, 1931. (*b*) Pappenheimer, A. M.; Goettsch, M., and Jungherr, E.: Nutritional Encephalomalacia in Chicks and Certain Related Disorders of Domestic Birds, Bulletin 229, Storrs, Conn., Agricultural Experiment Station, 1939.

2. Dam, H.; Glavind, J.; Bernth, O., and Hagens, E.: Nature, London **142**: 1157, 1938.

3. Waddell, J., and Steenbock, H.: J. Biol. Chem. **80**:431, 1928.

what sporadically, in spite of efforts to make the incidence more uniform. Since the condition appears to be identical with that described by Pappenheimer and Goettsch, the data concerning it obtained in these experiments have been collected and will be presented in this article, along with other information secured in supplementary experiments designed to give more information regarding the cause and prevention of the condition.

METHODS AND MATERIALS

The ration used in these feeding experiments (designated B. R. 152) consisted of: ground yellow corn, 46 parts; wheat bran, 13; wheat middlings, 13; alfalfa meal, 5; meat scrap, 10; dried skim milk, 10; salt, 1; cod liver oil, 2. Fresh batches of food were prepared at least once a week. Each batch was soaked in an ether solution containing 1 per cent (by weight of the food) of dissolved ferric chloride. After the mixture had stood for ten to twelve hours, the ether was evaporated on a steam cone at a temperature of 34.6 C. so that it could be reclaimed by condensation. The process was carried out in a closed system of glass containers so as to prevent access of the outside air. The method contrasts with the Waddell-Steenbock procedure [3] in that they allowed the ether to evaporate freely in the open air, *using no heat*. For each test a group of 15 to 20 newly hatched chicks was used. These were housed in a wire-floored pen and fed from the beginning of the experiment with the ration described. The addition of cod liver oil to the food was a precautionary measure to offset possible oxidation of vitamins A and D during treatment.

OBSERVATIONS

During Life.—During the first few weeks the chicks grew well and appeared to behave normally in all respects. The onset of the disease occurred with great suddenness during the third to fifth week and usually terminated rapidly in death, although in some cases the period of illness was prolonged. In a few cases slow recovery took place. The first reaction noted was a slight uncertainty in gait. This was followed by definite wobbliness, which ended in complete inability to stand or move about. At this last stage the affected birds were often found lying prone on the side with the head bent slightly backward, the legs extended more or less rigidly (fig. 1) and the wings spread outward. The rigidity of the legs was not due to paralysis, since the ordinary responses of kicking and attempting to rise could be obtained by disturbing the bird. Frequently the whole body was shaken by a violent tremor, which caused the legs to tremble visibly. Apart from these reactions there were no external signs of illness.

Autopsy.—Most experiments were carried on for a period of from five to eight weeks, since the condition usually manifested itself during the third to fifth weeks. All birds showing symptoms of the trouble were examined immediately after death, and the chicks remaining after an experiment had been terminated were killed and the brains carefully

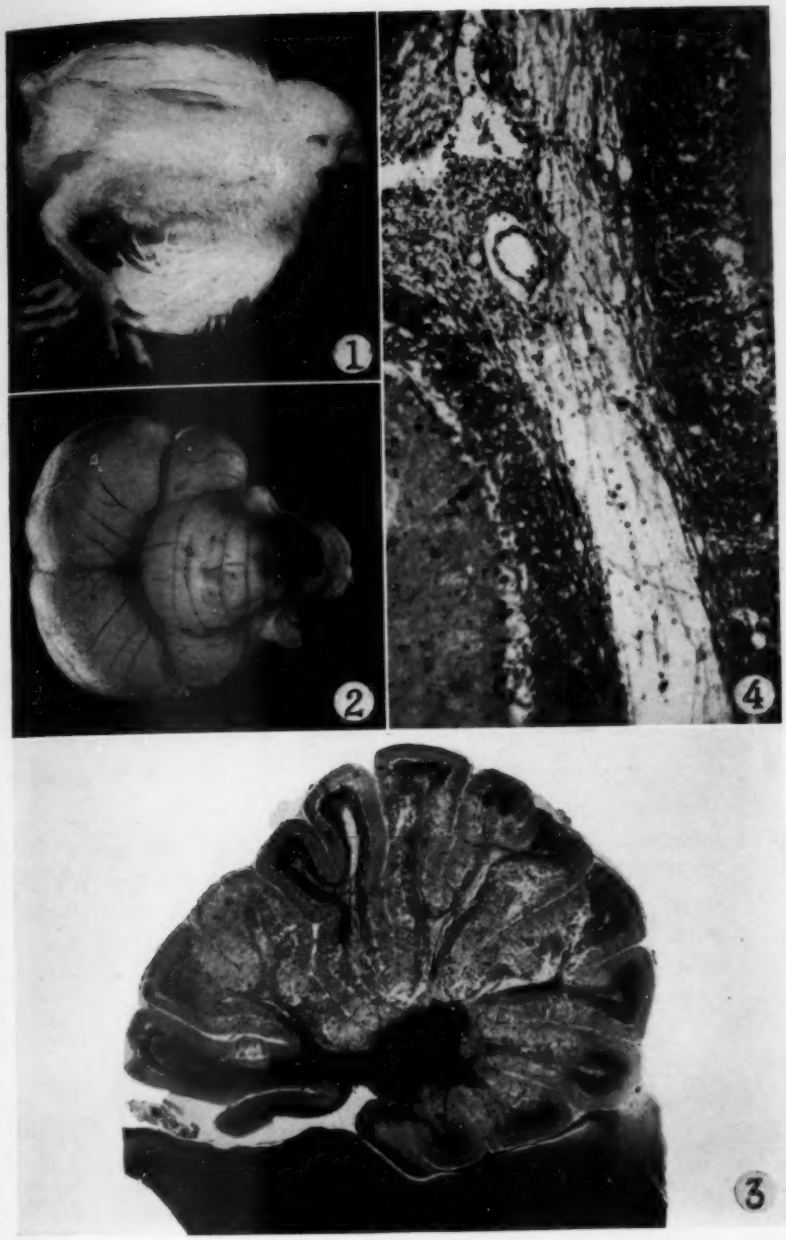

Fig. 1.—Typical prone position assumed by a chick when very seriously affected; reduced.

Fig. 2.—Dorsal view of the brain of the bird shown in A; × 2.

Fig. 3.—Longitudinal section of the cerebellum showing extensive degeneration; × 20.

Fig. 4.—Disorganization in the medullary strand of white matter; × 140.

examined. In general, the surface of the injured brain was marked by extensive blood spotting (fig. 2) or by a greenish yellow discoloration. In some cases the surface tissues were unaffected, thus obscuring extensive degenerate areas situated below the surface (fig. 3). Such cases were readily recognized when the brain was cut lengthwise. Most of the birds which died during the critical period showed gross brain lesions, and in some which survived until the experiment was terminated there were indications of an arrest of the brain degeneration. These last probably were the few chicks which showed indications of the disease for a few days and then spontaneous recovery. The number of birds showing brain injury varied considerably in different groups but on an average approximately one third were affected.

Histologic Studies.—The brains of the affected birds were removed at autopsy and fixed in 95 per cent alcohol for subsequent sectioning and histologic study. Routine staining methods, such as the use of methylene blue, Delafield's hematoxylin and eosin or Heidenhain's iron-alum-hematoxylin, were employed. The cerebellum of the brain was the region most frequently injured, but in some cases the cerebrum showed similar degenerative changes and in others lesions also occurred in the brain stem.

(*a*) Cerebellum: The degenerate areas of the cerebellum varied in extent from microscopic lesions to areas involving nearly the whole structure. Characteristically the following changes occurred:

1. Breakdown of the dendritic fibers of the outer molecular layer.
2. Degeneration of the large Purkinje cells (fig. 6).
3. Pyknosis and disintegration of the cells of the inner granular layer (fig. 7). The amount of destruction in this layer was often very extensive, involving nearly all of the cells in the affected area.
4. Extensive disorganization of the medullary strand of white matter (fig. 4).
5. Occurrence of thromboses in the minute capillaries in all layers of the convolutions (fig. 5).
6. Hemorrhage and seepage of blood through the disorganized tissues.
7. Changes in the staining reaction of the nerve cells in advanced cases. Thus, Purkinje cells, which ordinarily stain blue with methylene blue, lose this property and instead stain red with eosin in the methylene blue–eosin combination. This would appear to indicate a change in the acidity of these cells.

(*b*) Cerebrum: The number of chicks showing lesions in the cerebrum was proportionately quite small. Thus, only 5 of 42 diseased

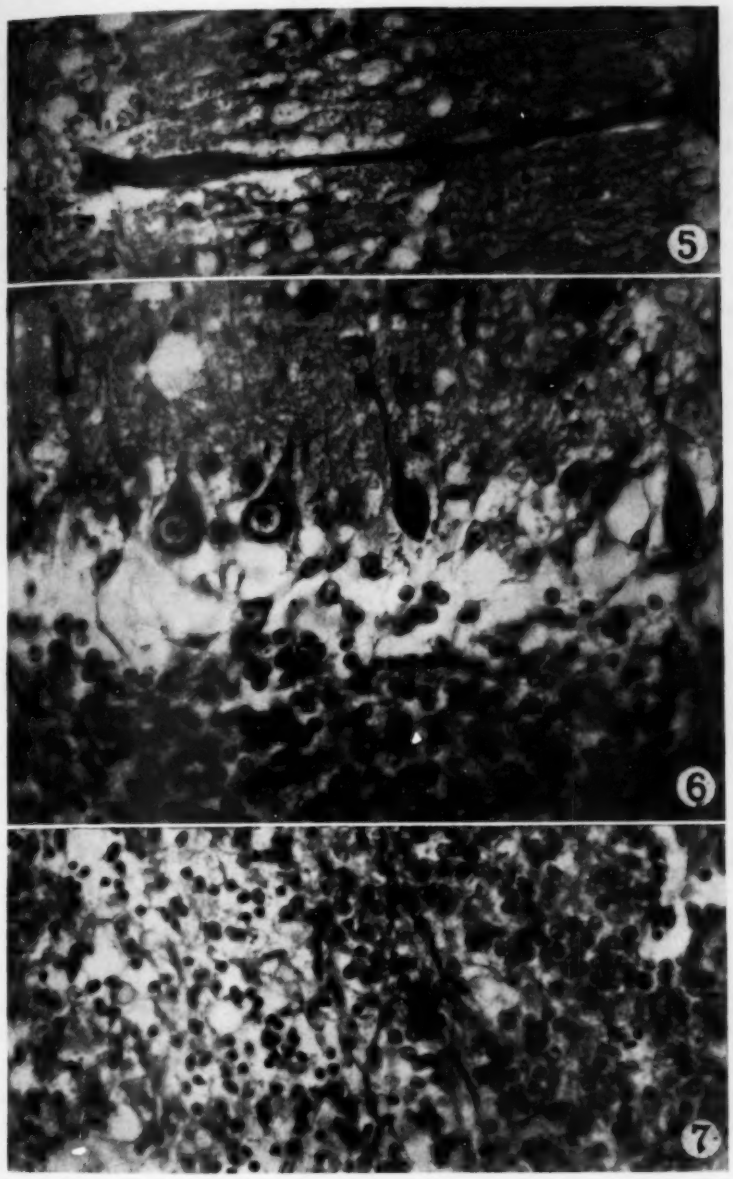

Fig. 5.—Thrombus in a capillary in a degenerate brain; × 207.

Fig. 6.—Disorganization of the dendritic fibers of the outer molecular layer and degeneration of the Purkinje cells; × 395.

Fig. 7.—Pyknosis and disintegration in the inner granular layer; × 395.

chicks listed in table 1 showed this reaction. The degenerate areas showed marked edema and contained large quantities of a watery fluid, which escaped on rupture of the overlying tissues. The degenerative changes, however, were similar to those observed in the cerebellum, notably necrosis of nerve cells, disintegration of fibers, pyknosis of nuclei, hemorrhage and capillary thromboses.

(*c*) Brain Stem: In the brain stem the degenerative changes were much more localized, usually involving a few individual nerve cells and the surrounding fibers. The lesions, therefore, consisted of many microscopic degenerate areas (fig. 3), which stained very lightly and gave the tissue a spotted appearance.

Diagnosis.—Careful study of the condition indicates beyond reasonable doubt that it is identical with the nutritional encephalomalacia of Pappenheimer and Goettsch. The external symptoms are alike in both outbreaks of the disease, and the histologic pictures are apparently identical in every respect. No attempt has been made to carry out a detailed cytologic study, as was done by Pappenheimer, for it seems sufficiently obvious from the histologic observations that the two conditions are identical.

CONTROL AND COMPARATIVE EXPERIMENTS

A number of control experiments were carried out for the purpose of comparing the typical disease with the effects produced by deficiencies in vitamins A, B complex and D as well as by methods of treating the food. Each of these vitamin deficiencies resulted in disturbances in the gait of the affected birds. In cases of B or D deficiency the reaction was quite distinctive, B deficiency being accompanied by the typical back somersault reaction and D deficiency by characteristic leg weakness. The reaction of the birds to vitamin A deficiency, however, was very similar to that occurring in chicks receiving the treated ration, but no gross lesions were found in the brains. The possibility that the use of ferric chloride or ether might have caused the condition was investigated by giving food to which ferric chloride had merely been added or which had been soaked in ether and from which the ether later had been evaporated in the usual manner. No reaction was produced in either case. In another group of experiments the food was treated by the original Waddell-Steenbock method,[3] i. e., without the use of heat, in order to find out whether the process of evaporating the ether had any effect. Finally the possibility that the kind of vitamin A-D supplement might be a factor was investigated by using sardine and halibut liver oil instead of cod liver oil. The results of this program of experimental work are presented in table 1.

The data given in this table indicate clearly that the degenerative condition of the brain cannot be ascribed to a deficiency of vitamin A,

B complex or D, since, although the gait of the birds is affected by a deficiency of any of these, the brain does not show the characteristic lesions. Furthermore, in experiments 1 to 4 the typical symptoms developed in some chicks in each group, although it was shown conclusively in other experiments that vitamins A and D were available in more than ample quantity. It is also evident from this same group of tests that the source of vitamins A and D, i. e., whether in the form of cod, sardine or halibut liver oil, was not a factor in the results, since the condition occurred in chicks receiving each of these supplements. Experiments 6 and 7 show also that ferric chloride and ether were not in themselves responsible for the condition. On the other hand, experi-

TABLE 1.—*Incidence of Nutritional Encephalomalacia in Relation to Diet*

Experiment	Diet *	Trials	Chicks	Gait of Affected Birds	Chicks with Brain Lesions
	Experimental:				
1	B. R. 152 Mod. Waddell + C. L. O.....	2	50	Erratic	20
2	B. R. 152 Mod. Waddell + S. L. O.....	2	35	Erratic	8
3	B. R. 152 Mod. Waddell + H. L. O.....	2	40	Erratic	10
4	B. R. 152 Mod. Waddell + S. L. O.....	1	20	Erratic	4
	Control:				
5	B. R. 152 Untreated + S. L. O.....	2	33	Normal	0
6	B. R. 152 Untreated + $FeCl_3$.....	2	30	Normal	0
7	B. R. 152 Ether treated only.....	1	10	Normal	0
	Comparative:				
8	B. R. 152 Waddell Treated + S. L. O....	2	30	Erratic	0
9	B. R. 152 Waddell Treated twice + S. L. O.....	1	20	Erratic	0
10	White Corn A Deficient.....	1	15	Erratic	0
11	White Corn A Deficient.....	1	20	Erratic	2
12	Synthetic B Deficient.....	1	20	Erratic + back somersault	0
13	D Deficient.....	1	20	Erratic + leg weakness	0

* In this column Mod. Waddell means the iron-treated ration from which the ether has been evaporated by heat. C. L. O., S. L. O. and H. L. O. stand for cod, sardine and halibut liver oil.

ments 8 and 9 by contrast with experiments 1 to 4 appear to indicate that the method of treating the food *with the use of heat* was associated with the trouble, since the condition failed to develop in the trials in which the original Waddell-Steenbock method was employed. The use of the latter method, however, was marked by an erratic gait which was very similar to that seen in the experimental animals. Since the typical lesions were never found in these birds, it is probable that a condition of vitamin A deficiency was involved and that the condition was not true nutritional encephalomalacia.

CURATIVE TESTS

In view of the results outlined and the strong presumption that vitamin E might be involved, a number of curative tests were carried out to ascertain whether wheat germ oil or some other substances would

be effective in preventing or curing the condition. The latter possibility was investigated by supplying corn oil, peanut oil and linseed oil. A summary of these experiments is given in table 2.

The results of these curative tests show that vitamin E affords decided protection against the brain degeneration but does not prevent it entirely. The experiments listed show a reduction in incidence from 29 per cent (42 of 145 birds) to 6 per cent (5 of 82 birds) following the use of wheat germ oil. Both corn and linseed oil also apparently arrest it in an early stage, so that no recognizable symptoms occur. It is interesting to note that with corn oil added to the food at a level of 1 per cent there were still considerable numbers of affected chicks but that the number was correspondingly decreased when the oil was

TABLE 2.—*Experiments with Curative Substances*

Diet *	Curative Substance	Chicks	Chicks Showing Lesions	Healed Brain Lesions	
				Cerebellum	Cerebrum
B. R. 152 Mod. Waddell + C. L. O.	Wheat germ oil	82	5	..	..
B. R. 152 Mod. Waddell + S. L. O.	E concentrate from wheat germ oil	15	2	..	..
B. R. 152 Mod. Waddell + S. L. O.	1% corn oil	20	9	..	..
B. R. 152 Mod. Waddell + S. L. O.	2% corn oil	20	3	1	..
B. R. 152 Mod. Waddell + H. L. O.	E. concentrate from wheat germ oil	15	3	..	..
B. R. 152 Mod. Waddell + S. L. O.	1 cc. corn oil orally twice weekly	20	0	1	..
B. R. 152 Mod. Waddell + S. L. O.	2% peanut oil	20	1	2	3
B. R. 152 Mod. Waddell + S. L. O.	1 cc. peanut oil twice weekly	20	0	2	..

* The abbreviations are explained in the footnote to table 1.

increased to 2 per cent. The chicks, however, did not thrive well, presumably because of excessive amounts of oil. Accordingly, in another test the oil was administered orally, 1 cc. per chick twice a week for the first two weeks and then three times a week. Among these chicks there was no mortality due to brain degeneration, but at autopsy two chicks showed lesions which had apparently been healed in an incipient stage. In addition to these curative tests, the effectiveness of corn oil was rather severely tested on a number of chicks from other groups. These had already reached the prone stage and would ordinarily have been considered doomed. They were given massive doses of corn oil, 1 cc. mornings and evenings, and 6 of 10 recovered. Two weeks afterward they appeared perfectly normal. At autopsy, however, their brains showed healed lesions.

COMMENT

It appears to be evident from the results of the experiments which have been carried out that the brain degeneration described is identical

with the nutritional encephalomalacia described by Pappenheimer and Goettsch. Like this disease, it is not due to a deficiency of vitamin A, B complex or D, but the preventive action of wheat germ oil strongly suggests that vitamin E is involved directly or indirectly. The results parallel those reported by Pappenheimer and Goettsch almost exactly. These investigators secured a considerable degree of protection with wheat germ oil and more with corn and peanut oils, and preliminary experiments with alpha tocopherol have given them promising results. The value of alpha tocopherol has also been demonstrated by Dam and co-workers.[2] While the present experiments do not include any work with alpha tocopherol, some of this substance has been made available by Merck & Company, and its effectiveness is under test.

The present results have all been accumulated by the use of a ration treated with ferric chloride and ether. The methods employed, however, involved the evaporation of the ether by means of heat after prolonged soaking. It is a remarkable fact that when the food had been treated by the original Waddell-Steenbock method,[3] without the use of heat, brain degeneration did not occur. Pappenheimer and Goettsch have obtained the same results using this method. Both methods, however, appear to be equally effective in bringing about the destruction of vitamin E, for in other experimental work carried out by me, with rats, it has been found that sterility of the female and testicular degeneration in the male can be readily produced by feeding the rats the heat-treated ration. It would appear, therefore, that vitamin E is destroyed during the soaking process, while the food is in contact with the cold ether-iron solution. Hence it must be assumed that some further reaction is set up by the action of heat after the destruction of vitamin E has been accomplished. Furthermore, it also appears essential that vitamin E be destroyed prior to heating, because when the food is merely soaked in ether (without ferric chloride) before heating, the brain disorder does not occur and the chicks appear to thrive exceptionally well. Since this is the case the production of nutritional encephalomalacia would appear to be due to several factors, one of which is the lack of vitamin E. This possibility affords some explanation of the erratic nature of the disease and the difficulty of preventing it when different vitamin E–deficient diets are used.

SUMMARY

In the course of feeding experiments using a modification of the Waddell-Steenbock ether-iron treatment of a natural ration, the modification consisting in the use of heat to evaporate the ether, a condition of brain degeneration identical with nutritional encephalomalacia was produced in young chicks.

The characteristic effect of the disease is the degeneration of the cerebellum or cerebrum, accompanied by loss of muscular control in the legs. Death usually occurs, although spontaneous recovery sometimes takes place.

The peripheral nervous system is not affected, and true paralysis does not occur.

The condition does not develop when the ether is allowed to evaporate spontaneously after the food has been treated.

It appears regularly when the food is heated to expel the ether. The heating of the food is therefore regarded as a factor in causing some other change in the food in addition to the destruction of vitamin E.

Curative trials indicate that the condition is not due to a deficiency in vitamin A, B complex or D.

Certain oils, namely, wheat germ, corn and peanut oils, appear to have protective value and hence contain some substance needed to prevent the disease.

CONCLUSIONS

Nutritional encephalomalacia can readily be produced in young chicks reared on a natural ration treated with ferric chloride in ether to destroy vitamin E *if heat is used to* evaporate the ether. The disease does not occur when the ether is allowed to evaporate spontaneously in the cold. The destruction of vitamin E by ferric chloride is a necessary preliminary, since the use of ether alone in treating the food and its subsequent evaporation with heat produce no results. Hence it is concluded that nutritional encephalomalacia is caused by at least two conditions occurring simultaneously, namely:

1. A deficiency of vitamin E.
2. The lack of some other heat-labile substance or substances, or the failure or inability of the animal to utilize some substances, if present, under conditions of vitamin E deficiency.

ERYTHROPHAGOCYTOSIS IN CHICKS REARED ON A VITAMIN E-DEFICIENT RATION SUPPLEMENTED WITH HALIBUT LIVER OIL

F. B. ADAMSTONE, PH.D.
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Previous experimental work with young chicks reared on a ration treated with ferric chloride in ether to destroy vitamin E (Waddell and Steenbock [1]) produced a characteristic reaction involving destruction and invasion of normal tissues by a reticular type of tissue and excessive accumulation of lymphocytes in the affected organs (Adamstone [2]). In this earlier work the treated food was supplemented with cod or sardine liver oil in order to offset any possible destruction of vitamins A and D which might have occurred during treatment. These oils proved to be interchangeable, since identical results were obtained with both of them, but in the present experiments the mere substitution of halibut liver oil for the cod or sardine oil has been accompanied by an entirely different reaction.

EXPERIMENTAL METHODS

The preliminary part of this study was begun with the collaboration of Dr. H. J. Sloan, now at the University of Minnesota. Dr. L. E. Card, head of the Poultry Division of the University of Illinois, placed at our disposal both equipment and experimental animals.

As in former experiments, the chicks were confined, from the time of hatching, in wire-floored brooder pens and fed continuously on the treated food. The chicks were from the same white leghorn stock as those used previously, and the food except for the vitamin A-D supplement was also the same as that used on former occasions. It consisted of basal ration 152, made up as follows: ground yellow corn, 46 parts; wheat bran, 13; wheat middlings, 13; alfalfa meal, 5; meat scrap, 10; dried skim milk, 10; salt, 1. The food was treated with a solution of ferric chloride (1 per cent by weight of the food) in ether, and after twelve hours' soaking the ether was evaporated off by heat. Halibut liver oil was added to this diet to the extent of 0.4 or 0.5 per cent. The original experiment (lot 74) was repeated with four different groups of chicks in order to investigate various phases of the problem, as shown in the accompanying table. The experiments listed in the table had the following objectives: For lot 76 the food was supplemented with a vitamin E concentrate in an attempt to prevent the condition produced by use of the treated food. Lot 87 received untreated food supplemented with halibut

From the Department of Zoology, University of Illinois.

1. Waddell, J., and Steenbock, H.: J. Biol. Chem. **80**:431, 1928.
2. Adamstone, F. B.: Am. J. Cancer **28**:540, 1936.

liver oil in order that they might serve as normal controls. They survived the experiment in good condition. In lot 88 the time of onset of the experimental condition and also the effect on the spleen and bone marrow were under investigation. For lot 89 a standard medicinal grade of halibut liver oil, purchased for the purpose, was used as the dietary supplement rather than the experimental oil in order to find out whether there was any difference between these grades.

The halibut liver oil used in lots 74, 76, 87 and 88 was an experimental grade supplied by Mead Johnson & Company. It was rated as supplying 65,000 U. S. P. units vitamin A and 1,100 units vitamin D per gram. The medicinal oil (used for lot 89) was slightly less potent, supplying 50,000 U. S. P. units vitamin A per gram and not less than 850 units vitamin D per gram.

In addition to the experiments enumerated, several tests carried out in connection with other phases of the work are of interest: 1. A series of 20 chicks were fed for eight weeks on a diet in which ferric chloride was merely added to the untreated ration. No harmful effects were observed. 2. A series of 15 chicks were fed food which had been soaked in ether and from which the ether later had been evaporated in the usual manner. These chicks survived the experiment in as good condition as normal controls.

Summary of Experiments Relating to Use of Halibut Liver Oil with Vitamin E–Deficient Ration

Lot	Chicks	Food	Supplement
74	18	B. R. 152 treated with $FeCl_3$	0.4% halibut liver oil
76	18	B. R. 152 treated with $FeCl_3$	0.4% halibut liver oil and vitamin E concentrate
87	20	B. R. 152—untreated control.	0.4% halibut liver oil
88	25	B. R. 152 treated with $FeCl_3$	0.4% halibut liver oil
89	32	B. R. 152 treated with $FeCl_3$	0.5% halibut liver oil (medicinal)

EXPERIMENTAL RESULTS

Condition of the Birds.—A daily check was made on the condition of the chicks as the experiment progressed, and sick or dead birds were removed for autopsy. The experiments on lots 74 and 76 were continued for three months, when all surviving birds were killed and examined. Superficially they all appeared to be in good physical condition except that their shanks and head furnishings were pale and their irises had a pale gray color. They had also maintained their weights fairly well. In the succeeding discussion particular attention will be paid to the chicks in these two groups, since the subsequent histologic studies were made largely on materials from them.

Autopsy Findings.—Of the 36 chicks which were started in lots 74 and 76, 2 died in the first few days of the experiment from unknown causes, 13 died early in the experiment, during the third to fifth week, as the result of cerebellar disintegration, and the remaining 21 survived until the time of autopsy. In all of the last group the liver was marked by numerous dark mahogany brown nodules, which occurred through-

out the whole organ and were visible on any cut surface (fig. 1). Frequently, the liver was swollen and friable, and large areas showed general dissemination of the brownish discoloration. Since all chicks had been decapitated and bled at the time of autopsy, it is evident that the brown spots could not represent accumulations of blood, as was at first suspected.

Although in the birds of the first two lots the spleen appeared quite normal, more particular attention was paid to it in experiment 88, but no evidence of abnormality or undue enlargement was found. The marrow of the long bones in these birds was also carefully examined. In most cases it was dark reddish brown and so firm that it could be removed from the split bone as a cylindric, rodlike piece. In the normal condition the marrow has a rather pale, reddish gray color and is quite soft.

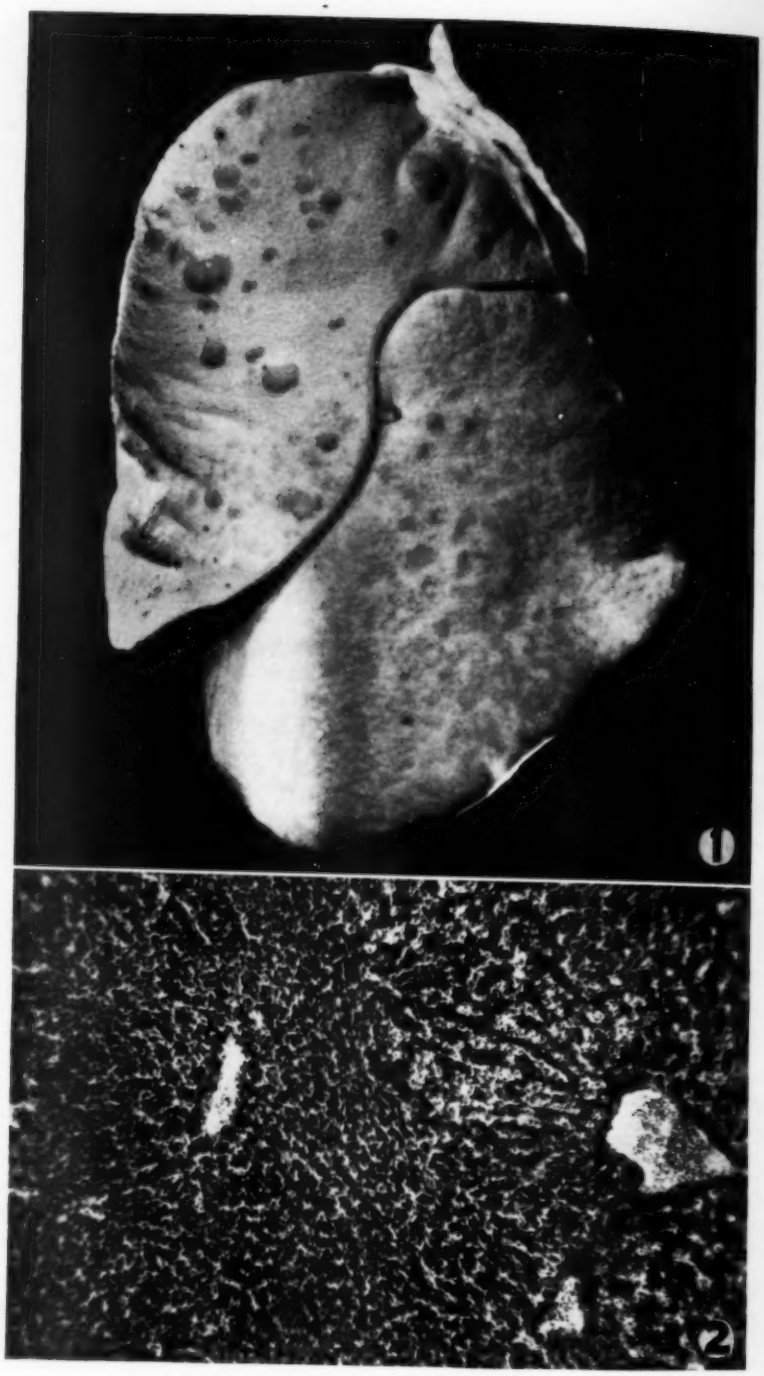
As to the time of onset of the abnormal condition, the lesions were small but unmistakable at eight weeks, after which they became progressively more pronounced. Furthermore, identical lesions were found both in birds receiving the experimental and in those receiving the medicinal oil.

Histologic Observations.—The liver, spleen and marrow tissues secured at autopsy were fixed in Bouin's solution; later, material from 25 chicks was sectioned. This included all specimens from lots 74 and 76. Standard staining methods,[2a] such as the use of Delafield's hematoxylin and eosin, Heidenhain's iron-alum-hematoxylin and picro-indigo-carmine, proved to be most satisfactory. In addition, some preparations were subjected to histochemical tests as described in the following paragraphs.

(*a*) Liver: Under low power of the microscope the lesions in the liver were very conspicuous because of the widening of the sinusoids. This produced a series of wide-radiating blood channels in the tissues immediately surrounding the larger blood vessels, while the unaffected tissue appeared much more compact (fig. 2). Detailed examination of areas such as these revealed the following conditions:

1. The individual hepatic cells in the lesions were mostly intact but were greatly enlarged, and their cytoplasm was finely vacuolated and often stippled with minute granules. Some degeneration of the liver cells had occurred, as shown by the disintegration of the cytoplasm. In most specimens the enlarged hepatic cells occurred in small, restricted areas around the blood vessels, but in occasional ones they were widely disseminated. These represented specimens in which the brown discoloration was general and the liver very friable.

2a. Lee, A. B.: Microtomist's Vade-Mecum ed. 10, Philadelphia, P. Blakiston's Son & Co., 1937.



Figures 1 and 2

(See legend on opposite page)

2. The sinusoids were usually gorged with blood cells, both erythrocytes and leukocytes being present in extraordinary numbers. Monocytes were the most abundant of the leukocytes, although in some specimens large accumulations of eosinophils were aggregated around some of the larger blood vessels. Kupffer cells, prominently anchored in the blood channels, were also exceptionally abundant, particularly in early stages of the condition. Both monocytes and Kupffer cells contained numbers of erythrocytes (figs. 3 and 4). The ingested erythrocytes varied widely in appearance; some, having only recently been ingested, were normal in appearance, while others showed all stages in disintegration of the nucleus and cytoplasm down to the final condition, in which they were merely ghost cells, represented by an oval vacuole in the engulfing phagocyte.

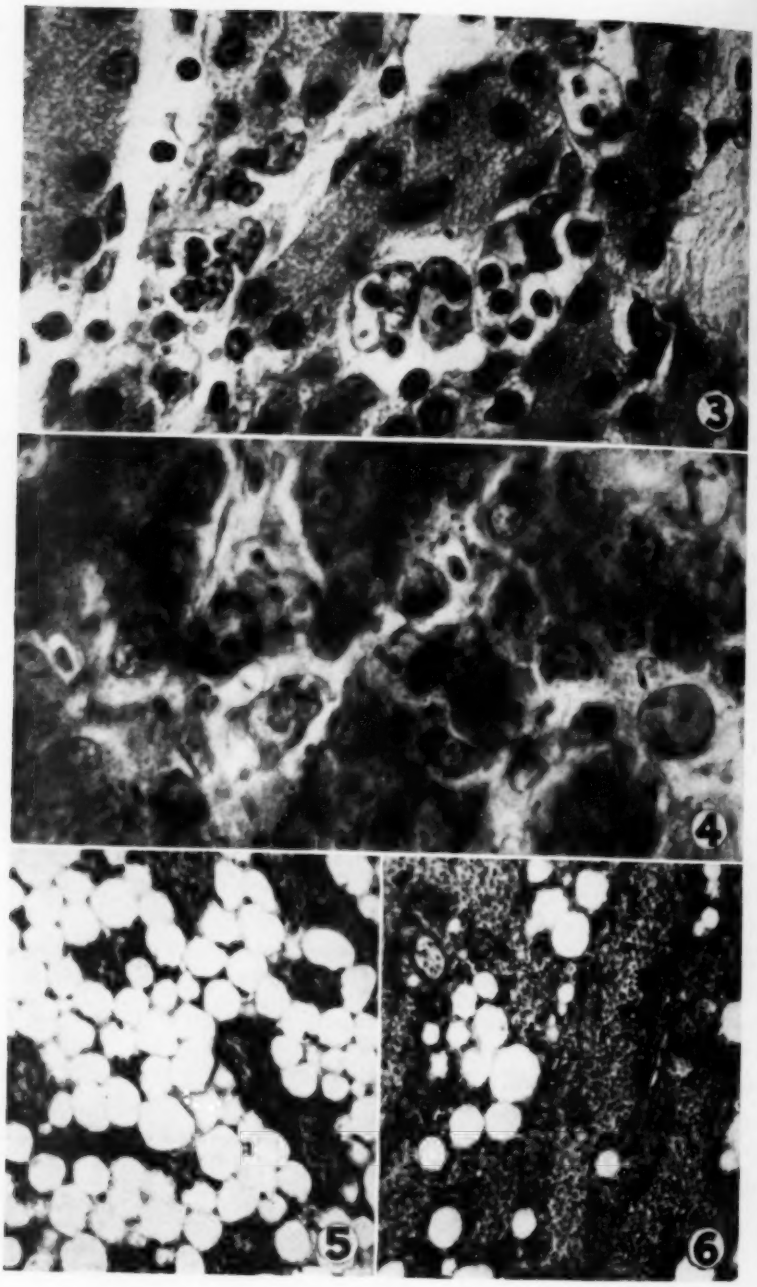
3. The cytoplasm of the hepatic cells usually contained minute brownish particles. In addition, larger, irregularly shaped, amorphous masses of an apparently similar material occurred in considerable abundance among the liver cells. This material was very prominent in cleared unstained sections and with dark field illumination caused the entire area of a lesion to appear as a brightly shining grayish patch. The minute granules in the liver cells were clearly visible, and brilliant points of light marked the larger particles. Apparently, therefore, the larger, amorphous masses and the minute granules in the hepatic cells were composed of some substance precipitated out in the tissue.

Nature of the Precipitate in the Liver Tissue: Since no precipitate had been encountered in tissues from chicks reared on the iron-treated diet supplemented with cod or sardine liver oils, the brown material was not thought to be an iron compound but was suspected of being an organic substance having some relation to halibut liver oil. Solubility tests showed that the material was quite unaffected by a long list of common solvents, including water, various alcohols, chloroform, ether, acetone, carbon disulfide, benzene, xylene, alkalis and glacial acetic acid. Strong solutions of mineral acids, such as hydrochloric, readily removed the material, but dilute solutions acted very slowly. Finally, after a number of other tests had been made, tests for iron carried out on unstained sections gave the characteristic insoluble prussian blue precipitate with potassium ferrocyanide, and sections treated momentarily with a weak solution of hydrochloric acid and flooded with

EXPLANATION OF FIGURES 1 AND 2

Fig. 1.—Brown-spotted liver characteristic of chicks receiving halibut liver oil supplement along with iron-treated, vitamin E–deficient food; × 2.

Fig. 2.—Section of a portion of liver; × 140. The widened sinusoids characteristic of the lesions appear at the right and an unaffected area at the left.



Figures 3 to 6

(See legend on opposite page)

a solution of ammonium thiocyanate gave the typical blood red color indicative of ferric iron. Slides showing the prussian blue reaction were made into permanent mounts. Microscopic study of these preparations showed a deposit of minute blue granules in the liver cells (fig. 5). The granules varied in size, abundance and distribution within the cell, and when examined with the oil immersion lens they were seen to be mostly short rodlike bodies. Larger, irregular masses were scattered within and among the liver cells, and both monocytes and Kupffer cells were often heavily charged with the precipitate. These preparations gave a much clearer picture of the conditions existing in the liver sinusoids. The extraordinary mobilization of monocytes and Kupffer cells was clearly evident, as well as the extensive phagocytosis of erythrocytes. In general, moreover, the phagocytes were confined to the liver sinusoids and were not, except in 1 or 2 cases, unusually abundant in the larger blood vessels.

(*b*) Spleen: The gross indication that no changes had occurred in the spleen was substantiated by histologic studies of sectioned material from 12 birds. In all of these sections the spleen appeared quite normal. In addition, chemical tests failed to show deposits of iron in any significant amounts.

(*c*) Bone Marrow: The histologic picture presented by the marrow, on the other hand, showed progressive replacement of the adipose tissue which is normally present by greatly increased amounts of myeloid tissue in active stages of hemopoiesis (figs. 5 and 6).

COMMENT

Source of the Iron Deposits in the Liver.—At first sight the extensive deposits of iron precipitate in the liver cells would appear to be due to the excess iron in the food. This explanation becomes untenable when it is recalled that the use of the same diet supplemented with cod or sardine liver oil was not accompanied by this reaction. This point was checked by making the potassium ferrocyanide test for iron on tissues from previous experiments in which these supplements had

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EXPLANATION OF FIGURES 3 TO 6

Fig. 3.—Portion of a lesion under high magnification showing phagocytosis of erythrocytes; × 520. The widened sinusoids and enlarged liver cells are evident.

Fig. 4.—Iron precipitate in the liver cells as shown by treatment with potassium ferrocyanide; × 520. The iron is indicated by small black dots.

Fig. 5.—Section of marrow from a long bone in a very early stage of the condition; × 207.

Fig. 6.—Section of marrow from a long bone at an advanced stage of the condition; × 207.

been used. These tissues (Adamstone [2]) were often heavily infiltrated with lymphocytes in contrast to the present material, in which both monocytes and Kupffer cells were unusually abundant. Both monocytes and Kupffer cells, moreover, contained disintegrating erythrocytes, from which the precipitated iron apparently originated as a breakdown product, probably in the form of hemosiderin.

Diagnosis.—Although no extensive studies of the blood have been carried out as yet, the histologic picture suggests an anemia. Several characteristic symptoms are present, including the loss of color in the shanks and head furnishings, the deposit of iron in the liver, the destruction of erythrocytes and the change in the myeloid tissue of the long bones. The last indicates exceedingly active hemopoiesis in an attempt to produce erythrocytes rapidly enough to replace those destroyed by phagocytosis. Obviously, therefore, the condition is not due to lack of iron or of the ability to produce erythrocytes and must be regarded as a secondary reaction.

Difference in Oil Supplements.—The results of the present experiments are so greatly different from those obtained when cod or sardine liver oil was used that there is obviously some distinct difference between those oils and halibut liver oil. Both reactions, however, involve injury and destruction of certain tissues and unusual proliferative activity on the part of others. When cod or sardine liver oils were used, tissues of nearly all visceral organs were susceptible to injury and replacement by a highly undifferentiated reticular tissue, which often showed destructive activity and would proliferate far in excess of the amounts needed to repair damage in the organs involved. This reaction was usually accompanied by excessive accumulations of lymphocytes. In the present instance, in which halibut liver oil has been used, the damage appears to be confined to the liver tissues and to the erythrocytes. The accompanying increase in monocytes and Kupffer cells is apparently a protective reaction to rid the body of injured erythrocytes, and the proliferation of myeloid tissue, an effort to compensate for the destruction of the red blood cells. The damage to the liver cells also appears to be related to the destruction of the red blood cells. The two reactions, therefore, are quite different.

Cause of the Reaction.—The question as to why the use of halibut liver oil should be followed by a reaction different from that accompanying the use of cod or sardine liver oil is not yet answerable, although there may be some relation to the fact that vitamin D occurs in several distinct forms, as shown by Bills.[3] It is clear, however, that vitamin E deficiency alone will not explain the result, because the deposit of iron can be produced with equal ease when vitamin E is restored to

3. Bills, C. E.: J. A. M. A. **108**:13, 1937.

the diet, provided the food has been treated with ferric chloride. Also, it does not occur in chicks reared on the normal diet supplemented with halibut liver oil. Hence it seems apparent that the treatment of the food with ferric chloride in ether and the use of halibut liver oil are necessary factors which enter into the reaction at some point. The most obvious conclusion, therefore, is that the process of treating the food or the occurrence of further chemical changes in the food attendant on the destruction of vitamin E (and possibly its associated antioxidants) results in the destruction of some substance besides vitamin E. If this is the case, cod or sardine liver oil apparently supplies the deficiency, whereas halibut liver oil does not. It must also be postulated that the resultant injury to the red blood cells is so severe that they must be removed from the circulation.

SUMMARY

Prolonged feeding of newly hatched chicks on a ration treated with ferric chloride in ether to destroy vitamin E but supplemented with halibut liver oil produced a condition having many characteristics of an anemia. It was marked externally by a gradual loss of color in the shanks and head furnishings and by the development of a pale gray color in the irises.

At autopsy the liver was frequently found to be much swollen and very friable and marked by dark mahogany brown spots. The marrow of the long bones was quite firm and dark red. No change had occurred in the spleen.

Histologic studies showed that the brown areas in the liver were characterized by a widening of the sinusoids, an enlargement of the liver cells and an unusual mobilization of monocytes and Kupffer cells. The increase in the number of phagocytes was accompanied by extensive destruction of the red blood cells.

The destruction of red blood cells was in turn accompanied by deposition of an iron compound, probably hemosiderin, in the form of small granules in and among the liver cells and also in the phagocytes.

In the marrow of the long bones there was a decrease in the amount of adipose tissue with a corresponding increase in the myeloid elements, presumably to compensate for the destruction of the red blood cells in the liver.

The cause of the phagocytosis of erythrocytes is not clear, but it is suggested that treatment of the food destroys not only vitamin E but also some other substance essential to the red blood cells which is available in cod or sardine liver oils but is not accessible in halibut liver oil. Hence, while the destruction of vitamin E is apparently a necessary factor in the reaction, the condition cannot be prevented by supplying vitamin E after the food has once been treated.

HISTOLOGIC EVIDENCE INDICATIVE OF THE NATURAL OCCURRENCE OF VITAMIN E DEFICIENCY IN THE CHICK

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In laboratory class work in embryology it had been found occasionally that spontaneous hemorrhage occurred in chick embryos when these were removed from the shells. At first rough treatment was considered to be the cause of this reaction, but it was soon apparent that the explanation was not satisfactory, for hemorrhage often occurred with the most careful manipulation, while other chicks, more roughly handled, failed to bleed. In addition to this, experience in injecting the embryonic blood vessels showed that although the blood vessels were very delicate they possessed a remarkable degree of elasticity and did not readily break. Since a similar hemorrhagic condition had been found in vitamin E–deficient chick embryos (Adamstone [1]), specimens were collected in the laboratory, whenever found, for sectioning and later histologic study.

METHOD

The material was fixed in Bouin's fluid and mounted as serial sagittal sections 10 to 14 microns thick. Slides were stained with Delafield's or Heidenhain's hematoxylin. Seventy-two hour chicks were obtained most frequently, since this was the stage used for the laboratory work. This stage fortunately was one of the most desirable for the study of the hemorrhagic condition in vitamin E–deficient embryos.

HISTOLOGIC DIAGNOSIS OF VITAMIN E DEFICIENCY IN THE CHICK EMBRYO

Histologic study of vitamin E–deficient embryos (Adamstone [1]) showed that hemorrhage occurred regularly in these embryos and that the site of hemorrhage was usually marked by small cells of a distinctive type grouped about the rupture in rosette-like clusters. They could be readily recognized by their densely staining pyknotic nuclei and clear cytoplasm (figs. 1 and 2).

HISTOLOGIC OBSERVATIONS ON SUPPOSEDLY NORMAL EMBRYOS

Histologic study of the embryos collected in the laboratory showed not only hemorrhage but also the presence of groups of cells which appeared to be identical with those seen in vitamin E–deficient embryos.

From the Department of Zoology, University of Illinois.

1. Adamstone, F B.: J. Morphol. **52**:47, 1931.

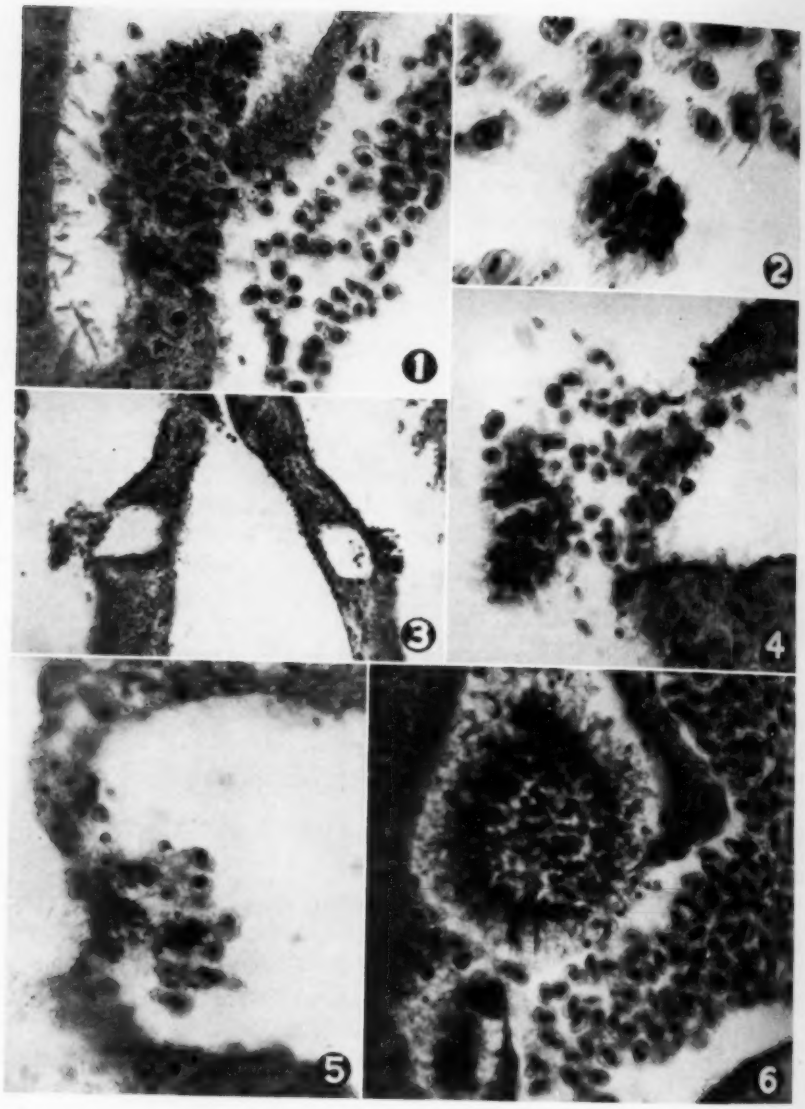
These cells, as in vitamin E–deficient embryos, usually occurred in rosette-like clusters, with their densely staining pyknotic nuclei pointed toward the center of the group and their clear cytoplasm extending outward. The shape of the nucleus varied from pear shape to elongate oval. The similarity of the clusters of diagnostic cells in vitamin E–deficient chick embryos to those found in the supposedly normal embryos can readily be seen in the photographs illustrating them. Figures 1 and 2 show conditions in embryos known to be from vitamin E–deficient hens. Figures 4, 5 and 6 illustrate the characteristic groups of pyknotic cells in 3 supposedly normal chicks in which hemorrhage

Location of the Site of Hemorrhage and Occurrence of Diagnostic Cells

Embryo	Age, Hours	Site of Hemorrhage	Diagnostic Cells
1	72	Anterior cardinal vein	Present
2	72	Right allantoic artery	Present
3	72	Not located	Present
4	72	Right common cardinal vein	Present
5	72	Left postcardinal vein	Present
6	48	Dorsal aorta	Present
7	72	Left vitelline vein	Present
8	48	Dorsal aorta	Present
9	72	Right vitelline vein	Present
10	72	Not located	Present
11	48	Right and left vitelline artery	Present
12	72	Not located	Present
13	72	Left common cardinal vein	Present
14	72	Atrium; left common cardinal vein	Present
15	72	Bulbus arteriosus; left vitelline vein	Present
16	72	Meatus venosus; sinus venosus	Present
17	72	Atrium; sinus venosus	Present
18	72	Left anterior cardinal vein	Present
19	72	Right vitelline artery	Present
20	72	Meatus venosus; right common cardinal vein	Present
21	72	Right common cardinal vein	Present
22	72	Not located	Not found
23	72	Meatus venosus	Present
24	72	Left vitelline artery	Not found
25	72	Sinus venosus	Present

had occurred. This evidence strongly suggests that the two conditions are identical.

Altogether a total of 25 hemorrhagic embryos have been sectioned and examined. This represents an incidence of possibly 2 to 3 per cent, but the figure is frankly an estimate. The location of the actual site of hemorrhage varied in these embryos, but it was found in 21 of the 25 specimens. In 20 of these 21 specimens, and in 3 of the remaining 4, groups of pyknotic cells were recognized even though the rupture could not always be located. In the two remaining cases the cells were not found, and hence it may be assumed that some cause other than that existing in the 23 embryos may have been responsible for hemorrhage. The data obtained from examination of these embryos are summarized in the accompanying table. From these data it is apparent that the site of hemorrhage is highly varied and that in some embryos several ruptures occur.



FIGURES 1 TO 6

(See legend on opposite page)

COMMENT

Since the first announcement of the discovery of vitamin E by Evans in 1923, and until comparatively recently, the effects of a deficiency of this vitamin have been regarded as an almost purely academic problem. This is due to the fact that vitamin E is widespread in its distribution in various foodstuffs and hence a deficiency would not ordinarily be expected to occur. In reviews of the subject by Mattill [2] and Mason [3] it has been pointed out that considerable evidence has already accumulated from the work of Vogt Möller, Watson and Tien, and Currie and Shulte indicating the therapeutic value of vitamin E in the reproductive processes of the females of various species of animals. Furthermore, incubation of the hen's egg is marked by two clearcut periods of high embryonic mortality. The early peak, which occurs between the second and the sixth day (Payne; [4] Byerly [5]), coincides with a period of high embryonic mortality in chicks from the eggs of hens which have been kept on a vitamin E–deficient diet (Adamstone [1]). Barnum [6] observed this correspondence and also suggested that vitamin E deficiency might be the cause. This view is further strengthened by the histologic evidence which has been presented here.

2. Mattill, H. A.: J. A. M. A. **110**:1831, 1938.

3. Mason, K. E., in Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939, p. 1149.

4. Payne, L. F.: J. Am. A. Instructors & Investigators Poultry Husbandry **6**:9, 1910.

5. Byerly, T. C.: Proc. Fourth World's Poultry Cong., 1930, p. 178.

6. Barnum, G. L.: J. Nutrition **9**:621, 1935.

EXPLANATION OF FIGURES 1 TO 6

Fig. 1.—Site of hemorrhage in a seventy-two hour vitamin E–deficient chick embryo showing clusters of darkly stained pyknotic cells associated with the rupture of the atrium; × 335.

Fig. 2.—Isolated groups of pyknotic cells from vitamin E–deficient chick embryo; × 722.

Fig. 3.—Rupture of both vitelline arteries in a supposedly normal seventy-two hour chick embryo (specimen 11 in table); × 119.

Fig. 4.—Clusters of typical pyknotic cells associated with rupture of the left vitelline artery seen in figure 3; × 335.

Fig. 5.—Clusters of typical pyknotic cells associated with rupture of the right common cardinal vein (specimen 4 in table); × 335.

Fig. 6.—Rupture of the allantoic artery. Smaller clusters of pyknotic cells may be seen apparently implanted in the neighboring tissue below (specimen 2 in table); ×335.

Significance of the Pyknotic Cells.—As in the earlier work, the significance of the pyknotic cells associated with hemorrhagic sites is not entirely clear. They may have some function in stopping hemorrhage. This finds support in the fact that they always accumulate at the site of hemorrhage. On the other hand, the interval of time between removal of the embryo, when hemorrhage occurs, and immersion in a killing fluid is so short that practically no time is available for the formation of such well formed clusters. It might also be expected that these cells would be found within the blood stream if their purpose were to stop hemorrhage. This is not the case, and many independent groups of them are found in the celom.

A second alternative, namely, that the cells have a destructive function, finds support in several considerations: the accumulation of the cells outside the break; the occurrence of two or more hemorrhagic sites in certain specimens (table; fig. 3), which is difficult to accept as a mere coincidence; the frequent occurrence of these cells in other parts of the celom, and the fact that independent groups often appear to have settled down and to have implanted themselves by eroding adjacent tissues (fig. 6).

Although little has been done regarding the effects of other vitamin deficiencies on the development of chick embryos, the effects of E deficiency seem sufficiently characteristic and the response to vitamin E therapy so clear that the conclusion drawn seems justified. Nevertheless, it is fully recognized that further evidence may necessitate a revision of this opinion, since other factors might possibly bring about the same reaction. However, the possibility that vitamin K is involved is rejected because extensive experience with chicks reared on the treated ration has not revealed any effect on bleeding or clotting of the blood.

SUMMARY

The evidence presented shows clearly that supposedly normal embryos show spontaneous hemorrhage and a histologic picture which is apparently identical with that encountered in vitamin E–deficient embryos. The conclusion seems warranted that vitamin E deficiency can occur in chick embryos under supposedly normal feeding conditions, although in most animals such a condition is regarded as rather improbable because of the wide distribution of vitamin E. However, it must be remembered that in the chick the developing embryo is dependent on the food materials, including vitamins, stored in the egg by the hen. In times of high egg production it is conceivable that some eggs may lack a sufficient store of certain vitamins if sufficient green food is not available. This being the case it would be expected that the embryo would show a reaction to the deficiency even though none was apparent in the adult.

Case Reports

ANEURYSM OF THE SINUS OF VALSALVA CAUSING CORONARY OCCLUSION

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Aneurysm of the sinus of Valsalva is rare. It usually occurs in men in the third and fourth decades of life; it is generally of syphilitic, sometimes of arteriosclerotic and more rarely of mycotic or of congenital origin.[1] Because most of the aortic sinuses occupy an intracardiac position, aneurysmal bulgings and burrowings can produce symptoms and complications not ordinarily associated with aneurysms arising higher in the ascending portion of the aorta. For this reason aneurysms of the sinuses of Valsalva should be set apart from those of the remainder of the ascending portion of the aorta. Burrowing into the myocardium of the atria, ventricles or interventricular septum, they frequently rupture. In the literature one finds general or specific references to aneurysms of the aortic sinuses that have ruptured into the pericardiac sac, the left atrium, the right atrium, the left ventricle, the right ventricle, the pulmonary artery and the superior vena cava or the mediastinum.[2] Previous obliteration of the pericardial sac may so alter events that, as in Sheldon's [3] case, the aneurysm erodes the wall of the chest to burst externally, or it may burst into the left pleural cavity, as in Shuster's [4] case. Rupture may likewise involve a combination of these sites, as in one of the cases collected by Smith,[5] in which rupture occurred into the left ventricle and into the pulmonary artery. Rupture does not necessarily occur, however, and instances are recorded in which the bulging of the aneurysm caused valvular stenosis, particularly by extrinsic pressure on the septal pulmonary sinus, or caused valvular insufficiency, particularly by dilatation of the aortic ring. The aneurysm in one of the cases reported by Snyder and Hunter [2] caused heart block by encroachment on the atrioventricular bundle. These observers reported another case which was most unusual in that there was an extension of syphilitic disease from an aneurysm of an aortic sinus to an adjacent coronary artery with the formation of a secondary aneurysm of the coronary artery and subsequent thrombotic occlusion of the latter.

In the case now presented the aneurysm of the sinus of Valsalva led to a termination not hitherto recorded.

From the Department of Pathology, McGill University.

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2. Snyder, G., and Hunter, W. C.: Am. J. Path. **10**:757, 1934. Ostrum, H. W.; Robinson, B. D.; Nichols, C. F., and Widmann, B. P.: Am. J. Roentgenol. **40**:828, 1938. Wright, R. B.: Arch. Path. **23**:679, 1937. Norris, J. C.: U. S. Nav. M. Bull. **30**:37, 1932. Vogelsang, T. M.: Urol. & Cutan. Rev. **34**:62, 1930. Higgins, A. R.: U. S. Nav. M. Bull. **32**:47, 1934.

3. Sheldon, J. H.: Lancet **1**:178, 1926.

4. Shuster, N. H.: Lancet **1**:507, 1937.

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REPORT OF A CASE

A 39 year old white man was admitted to the Royal Victoria Hospital Sept. 18, 1939. Three years prior to admission he began to have indigestion and heartburn, and in the last year he had experienced dyspnea on exertion. For the last six months he had been having attacks of precordial pain, and with them, weakness of the left arm. Three days before he entered, severe precordial pain developed and persisted. Approximately three hours prior to his admission he was seized by a severe precordial pain. He became short of breath and weak.

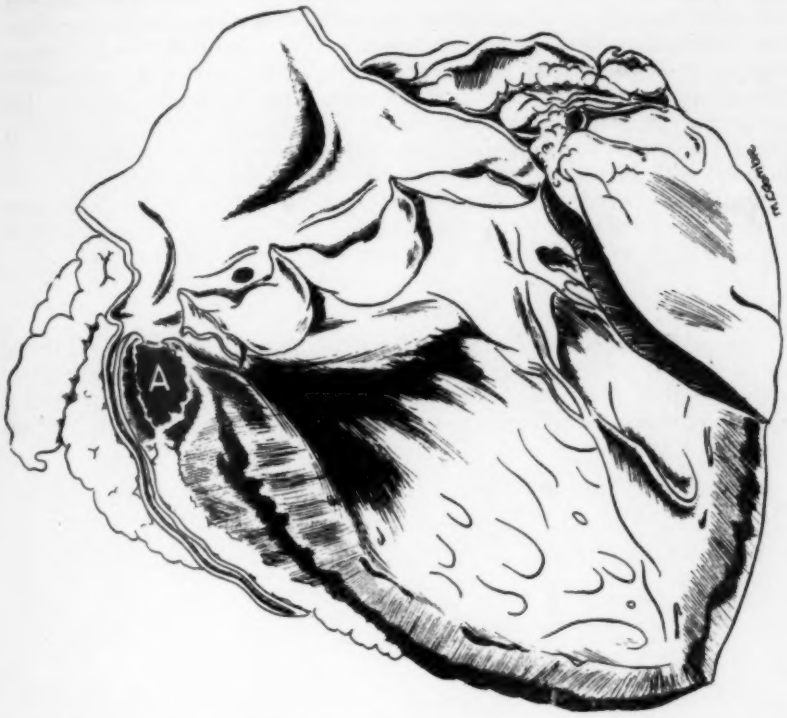
He was a somewhat obese patient complaining bitterly of severe continuous precordial pain. The skin was ashen in color, beads of perspiration stood out on the forehead, and the clinical picture was that of shock. The blood pressure was 100 systolic and 84 diastolic; the temperature, 97.6; the pulse rate, 96; the respirations, 25. There were signs of congestion at the bases of both lungs. The heart sounds were weak and distant; the pulse was irregular, with bigeminal rhythm. The white blood cell count was 27,000; the hemoglobin was 118 per cent. The urine contained a trace of albumin and numerous hyaline and granular casts. An electrocardiogram made seventeen hours after admission showed "an acutely negative T wave with an elevated RT interval in lead I and depressed ST intervals and slurred QRS complexes in leads II and III." These findings were interpreted as probably indicating "acute anterior coronary occlusion." A bedside anterior-posterior roentgenogram of the chest showed marked pulmonary congestion and did not show any definite cardiac or aortic abnormalities.

The patient's condition became steadily more grave. Because of the pain he required heavy sedation. Oxygen therapy and respiratory and cardiac stimulants were of no avail, and death occurred twenty-five hours after the patient's admission to the hospital. Blood for a Wassermann reaction had not been taken.

Autopsy (eight hours after death).—The right pleural cavity contained 500 cc. of clear fluid and the left 300 cc. The lungs were markedly congested and edematous. The other organs with the exception of the heart and aorta were essentially normal. The heart weighed 380 Gm. The valves were free from lesions. Opening into the anterior half of the left lateral aortic sinus was the orifice, 7 mm. in diameter, of a sacculated aneurysm that measured 2.3 cm. in its greatest length and 1.8 cm. in its greatest diameter. The orifice of the aneurysm was nearly circular and had somewhat rolled margins. The left coronary artery arose normally through a patent orifice, 4 mm. in diameter, which lay just above the mouth of the aneurysm. The two were separated, however, by 2 mm. of intact aortic intima. The intimal surface of the ascending aorta showed a number of elevated opaque plaques and wrinkled puckered areas consistent with the usual gross appearance of syphilitic aortitis. These areas descended into the aortic sinuses but did not narrow the coronary orifices or involve the cusps of the aortic valve.

The aneurysm, while entirely intracardiac, caused slight bulging of the external surface of the heart anteriorly near the origin of the pulmonary artery. The sac was directed downward into the myocardium of the anterior superior portion of the interventricular septum and left ventricular wall. The anterior wall of the aneurysm was found to lie just beneath the proximal portion of the left coronary artery and the origin of its branches, the ramus descendens and the ramus circumflex, so that the artery was stretched over the aneurysm. A sagittal cut through the coronary artery and aneurysm showed that the coronary artery was

separated from the aneurysmal cavity only by the wall of the aneurysm (figure). The bulging of the aneurysm toward the heart surface tended to enfold the overlying coronary, and the coronary lumen appeared to be markedly compressed from a point approximately 5 mm. from its orifice distalward for 2 cm. to the proximal portions of both the ramus descendens and the ramus circumflex. The intima of the proximal portion of the left coronary artery showed minimal arteriosclerosis but no thrombosis or intrinsic occlusion. The myocardium of the left ventricular wall in the areas supplied by the circumflex branch as well as by the descending branch



Semidiagrammatic drawing of the heart, which has been cut to show the relation of the left coronary artery to the aneurysm of the left lateral sinus of Valsalva. '*A*' indicates the sac of the aneurysm.

of the left coronary artery showed extensive alteration of color, there being a tigroid tannish red mottling in place of the normal homogeneous color.

Microscopic Examination.—The ascending aorta showed typical syphilitic mesaortitis. Sections from the wall of the sinusal aneurysm showed it to be lined by unorganized thrombus material, beneath which was hyalinized connective tissue containing only irregular and scanty fragments of muscle and elastic tissue. Irregular zones of fibroblastic proliferation were prominent, and there was heavy perivascular lymphocyte and plasma cell infiltration. The overlying coronary artery showed minimal arteriosclerosis but no arteritis or thrombosis. Sections of myocardium from the discolored areas described in the gross showed evidence of early

infarction in the form of deeper eosinophilic staining of fibers, loss of nuclei and striations, small zones of hemorrhage and exudation of polymorphonuclear leukocytes.

COMMENT

This case was one of an aneurysm of the left anterior sinus of Valsalva, which bore such a relation to the proximal portion of the left coronary artery that the latter was compressed by extrinsic pressure and myocardial infarction had occurred as a result. No blood for a Wassermann test was obtained, but the gross and microscopic appearance of the lesion and of the neighboring regions of the aorta clearly established the syphilitic nature of the condition. In the light of the findings at autopsy it seems entirely probable that the "heartburn" and "indigestion" the patient had experienced for three years were due to the aortitis. The precordial pain that developed six months before death was probably anginal and due to beginning compression of the left coronary artery. Finally, with increasing compression of the coronary artery, myocardial infarction occurred, probably coinciding with the sudden intensification of the precordial pain which the patient experienced a few hours before his admission to the hospital or twenty-eight hours before death.

SUMMARY

An additional case of discrete sacculated aneurysm of the left lateral aortic sinus is presented, in which compression of the left coronary artery with resulting myocardial infarction had occurred. This represents a hitherto unrecorded terminal possibility in cases of aneurysm of this type.

MALIGNANT MELANOMA CELLS IN THE BONE MARROW

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Apparently, no data are available as to the occurrence of malignant melanoma cells in bone marrow, although recent comprehensive reviews [1] and special studies on metastatic tumor cells in the bone marrow have contained ample descriptions of various other types of tumor cells.[2] Since the statement is generally made that widespread and extensive metastases in bone marrow are common in cases of melanoma,[3] the

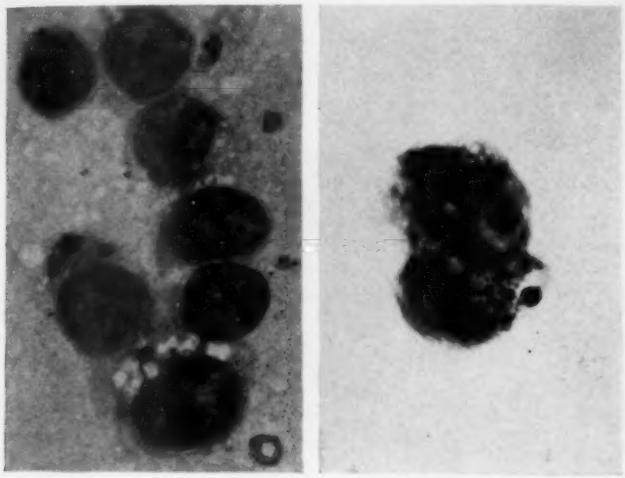

Fig. 1.—Melanoma cells as they appear in imprint preparations of marrow obtained by sternal puncture; Wright-Giemsa stain; left, × 1,000; right, × 1,160.

From the Department of Pathology and Bacteriology, School of Medicine, Louisiana State University, and Charity Hospital of Louisiana at New Orleans.

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2. (*a*) Fleischhacker, H., and Klima, R.: München. med. Wchnschr. **83**: 2051, 1936. (*b*) Markoff, N.: Deutsches Arch. f. klin. Med. **182**:47, 1938. (*c*) Rohr, K., and Hegglin, R.: ibid. **179**:61, 1936.

3. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, ed. 9-10, Berlin, Walter de Gruyter & Co., 1931, vol. 1.

finding of melanoma cells in the bone marrow on sternal puncture may prove of interest.

REPORT OF A CASE

A white housewife aged 60 years entered Charity Hospital of Louisiana at New Orleans Nov. 29, 1939, complaining of severe pains in the back. The right eye had been enucleated for malignant melanoma two years before. The patient was poorly nourished, appeared anemic and chronically ill, and presented signs of

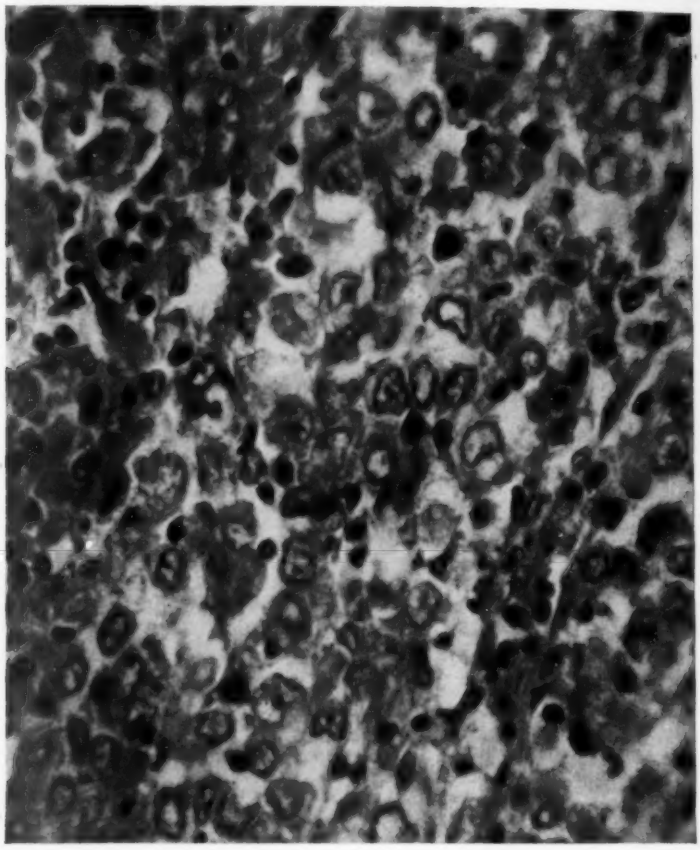

Fig. 2.—Malignant melanoma cells replacing normal bone marrow elements; Dominici's stain; × 600.

recent loss of weight. The blood revealed a marked microcytic and hypochromic type of anemia, for which the treatment subsequently instituted was without effect. All other routine laboratory examinations gave negative results, including roentgenologic examination of the lungs and of the long and flat bones.

Smears of bone marrow obtained by sternal puncture and stained according to the Wright and Giemsa technic revealed large atypical cells, measuring approximately 30 microns in diameter, with deep basophilic cystoplasm. Their nuclei were

large and showed a fine chromatin pattern and one or more conspicuous, disproportionately large, pale blue nucleoli. The pink parachromatin in the nucleus was sharply demarcated from the dark purple chromatin, which was arranged in a rather dense and irregular network. The cytoplasm of some cells contained a few large dark granules (fig. 1). The tentative diagnosis was diffuse metastases from malignant melanoma. The patient died of terminal pneumonia Jan. 23, 1940.

Postmortem examination revealed diffuse melanosarcomatosis. Pigmented and nonpigmented nodules of various sizes were found in the liver, spleen, peritoneum, mesenteric lymph nodes, heart, dura mater, meninges, kidneys and adrenal glands. The largest were 2 cm. in diameter. Similar but smaller nodules were found in the marrow of the ribs, sternum, dorsal vertebrae, and flat bones of the skull. The degree of infiltration varied, the heart and meninges revealing only a few scattered nodules, and the liver, right adrenal gland and bone marrow being densely infiltrated. The majority of the nodules were extremely cellular and showed only a small amount of stroma (fig. 2). The cells showed a tendency to form whorls, irregular nests or solid sheets.

Most of the tumor cells were oval or fusiform, and the small rim of cytoplasm contained varying amounts of yellowish brown to black granules. Mitotic figures were frequent. The nuclei were characterized by fine nuclear membranes with a small amount of chromatin arranged in fine granules; they contained one or more conspicuous nucleoli.

Examination of smear preparations of the rib marrow revealed the same changes as had been observed in the aspirated sternal bone marrow preparations.

COMMENT

Observers of metastatic cancer cells in bone marrow secured by puncture distinguish between large and small cells.[2] The small cells, which measure about 15 microns in diameter, are usually arranged in small groups and show narrow rims of blue cytoplasm and a finely reticulated chromatin network with occasional nucleoli. The large cells, which measure about 30 microns in diameter, show indistinct, narrow cytoplasm containing vacuoles and acidophilic granules. The nuclei are rich in chromatin and are finely reticulated and irregularly configurated. The opinion has also been expressed that the size of the cells depends on their arrangement and that cells arranged in cell groups are usually smaller than solitary cells.[2a]

The appearance of the metastatic tumor cells in bone marrow smears is not entirely characteristic. For this reason some authors[2c] have expressed the belief that a definite diagnosis of tumor cells can be made only when the cells are arranged in syncytia or in cell groups.

The tumor cells found in the sternal bone marrow in the case reported here closely resembled in many respects the large variety of metastatic tumor cells, the difference being that the cytoplasm of the melanoma cells frequently contained granules of dark pigment.

SUMMARY

A case is reported in which the finding of abnormal cells in the bone marrow on sternal puncture suggested diffuse metastases from malignant melanoma. Roentgenologic examination gave negative results, but the diagnostic assumption of metastases in the marrow was confirmed post mortem.

RUPTURED HUMAN FOLLICLE WITH OVUM IN SITU

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The exact mechanics of ovulation are of interest to the histologist and the endocrinologist alike. The process has been observed directly in the rabbit by Kelly.[1] Allen, Pratt, Newell and Bland described histologically several ruptured follicles and the ova that were recovered from the fallopian tubes.[2] Three or four theories of the mechanics of rupture are current: namely, that the increased secretion of the granulosa cells produces a follicular pressure that causes rupture; that endocrine principles stimulate the vascular plexus in the theca interna to exert a pressure on the follicle; that lysis of the superior surface of the follicle occurs under endocrine influence. That the process is partly vascular and partly mechanical is evident from Kelly's description of the successive rise and fall of a surface cone with hemorrhage into the translucent follicle before actual rupture occurs. To our knowledge, the only dissenting view is that of Strassmann,[3] who held that hemorrhage into the follicle is pathologic.[4] He, it appears, was defending his own hypothesis (colored by the influence of Aschoff's interpretation of the corpus haemorrhagicum) that a growth cone of the theca cells carries the follicle to the surface. He did not explain, however, the actual rupture itself. Hemorrhage into the follicle without rupture is a common observation in test animals, according to all investigators routinely carrying out pregnancy tests.

In the course of an investigation with various gonadotropins[5] we were fortunate enough to recover an ovum still retained in a ruptured follicle. We feel that this specimen is worth description, since it introduces a number of facts which bear on the various hypotheses cited.

REPORT OF A CASE

A white woman aged 22 was admitted to the University Hospital for sterilization. She was an inmate of an institution for the feebleminded. The menstrual cycle was normal and regular. On the fifth, sixth and seventh days of the cycle 1,400 units (Cole-Saunders) of equine gonadotropin was administered. Laparotomy was performed on the ninth day. The ovaries were slightly enlarged, and many small cysts were visible throughout both. On the superior border of the left ovary (fig. 1 *A*) there was an irregular raised reddish area about 1 cm. in diameter. In the center of this area there was a small pinkish-tinged coagulum. This whole area was excised. A biopsy specimen was also taken from the right ovary.

From the University of Georgia School of Medicine.

This investigation was aided by a grant from the Committee on Scientific Research of the American Medical Association.

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2. Allen, E.; Pratt, J. P.; Newell, Q. U., and Bland, L. J.: Contrib. Embryol. (no. 127) **22**:45, 1930; Am. J. Anat. **46**:1, 1930.
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5. Greenblatt, R. B., and Pund, E. R.: The Gonadotropins: A Clinical and Experimental Study, South. M. J., to be published.

Histologic Observations.—The general histologic picture of the ovary was normal. There were a few primary follicles and occasional secondary follicles, and in the sections examined from the left ovarian specimen there appeared three maturing follicles, the largest of which measured 3 by 6 mm. in the sections. There were five typical corpora albicantes and two small follicles undergoing recent atresia. In general the stroma and the blood vessels were normal. Of the three large follicles, one was normal throughout, but our sections missed the egg. The second was normal in all of its coats. The follicular cavity was 1.7 mm. in diameter, and the stratum granulosum was 150 microns wide. The egg was normal, the nucleus was within the center of the ovum, and the zona pellucida was well filled. The ovum measured 91 by 108 microns and fell within the group size recorded by Allen and co-workers. The only evidence of recent change in this follicle was an apparent increase in internal tension, expressed by an apparent stretching of the theca interna cells into continuous alinement, giving them all a fusiform character and producing a definite deeply staining band about the follicle. The superficial wall lay within 300 microns of the surface of the ovary. The third follicle was ruptured and partially collapsed, but the egg, surrounded by the granulosa cells of the cumulus, lay free near the orifice of the follicle. The follicle was roughly pyramidal, with the apex pointed outward, and was on the free surface of the ovary. The height of the pyramid was 1.3 mm. The base was 4 mm. in length and lay adjacent to the superficial wall of follicle 1. The lateral wall of the ruptured follicle lay adjacent to follicle 2 (fig. 1 *A*).

The wall of the ruptured follicle varied considerably in structure. At the peripheral pole the granulosa cells were reduced to a layer three to four cells deep but were still adherent at all points except at the aperture itself (fig. 1 *B*). On the superficial and lateral walls the theca interna, theca externa and surrounding stroma had suffered disintegration by interstitial extravasation. On the lateral wall this area was approximately 300 microns wide and evidently produced the infolding of the intact stratum granulosum into the follicle cavity (fig. 1 *B*). The veins were markedly congested and the capillaries dilated to 30 microns. The endothelium was still apparently normal in places, but direct continuity was apparent between the lumens and the tissue spaces.

On the deep basal wall a different condition prevailed. The stratum granulosum (100 microns thick) showed typical cells, with rounded, deeply staining spheroid nuclei in the surface layer. The deeper cells showed the streaming alinement, swelling and vesicular nuclei of initial luteinization. Mitoses were common. Invading fibroblasts, described by Allen and co-workers, were also in evidence. The blood vessels were dilated but intact. There was no extravasation. The theca interna cells were swollen, clear and rounded, indicative of early luteinization. They sometimes reached 30 microns in diameter (figs. 1 *B* and 2).

The basal half of the follicular cavity was remarkably free from both coagulum and red cells. But a definite plug made up of basophilic coagulum, discrete erythrocytes and partially dispersed and partially intact granulosa cells was attached at the rupture point (fig. 3). In this plug the egg was suspended and was situated in a recognizable cumulus. One Call-Exner body surrounded by its layer of granulosa cells was also included in the plug.

The ovum itself was seen in two sections. It was 75 microns wide and 50 broad. In figure 3 a dark spot appears at one pole. This does not, however, represent the polar spindle but a mechanically superimposed granulosa cell. In the second section two erythrocytes had been mechanically displaced over the ovum. Neither of the two sections showed the nucleus. Incidentally, mitotic figures were

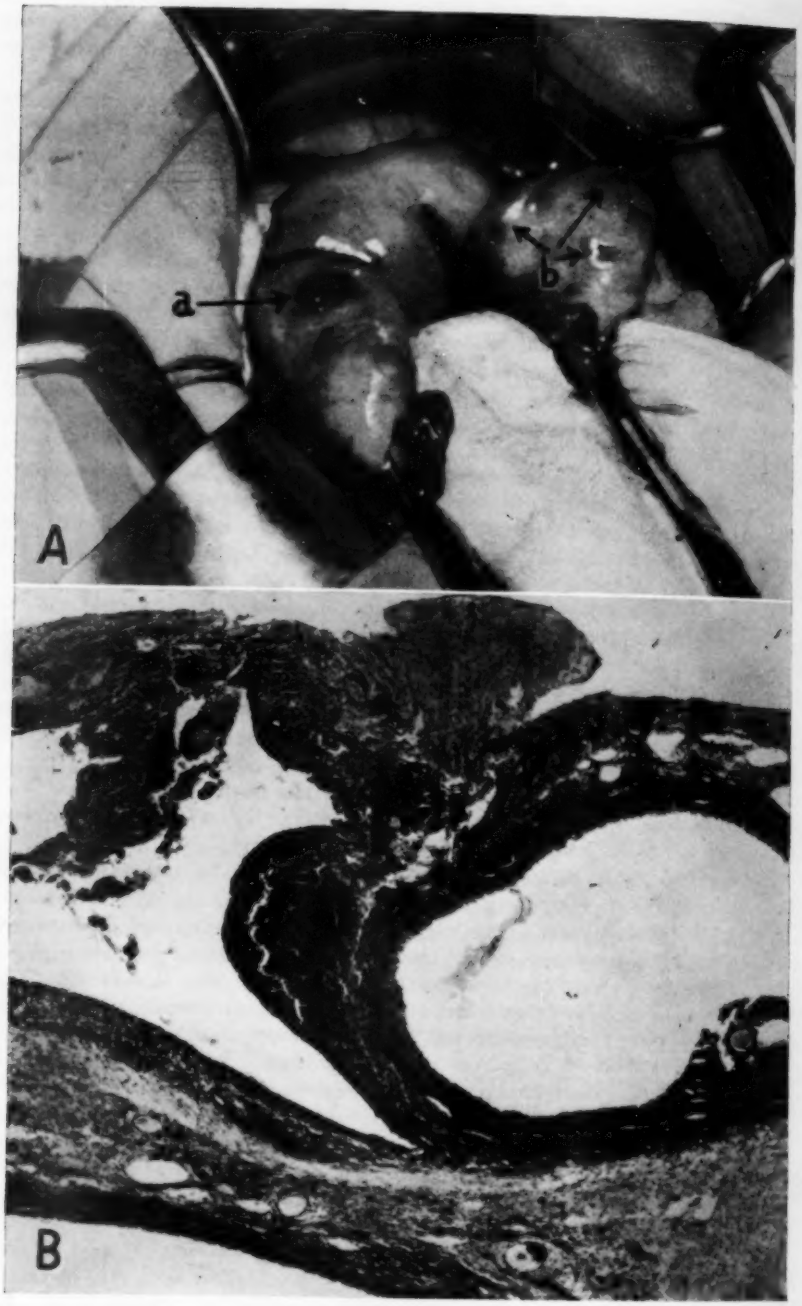

Figure 1

(See legend on opposite page)

not observed in any ovum, large or small. The ovum as seen in figure 3 practically fills the zona pellucida, but one or two vacuoles and a slight shrinkage space are to be noted.

From the general histologic picture it would seem that rupture of the follicle came about, not by jet burst of blood vessels into the cavity, but by disintegration of the outer wall by extravasation, somewhat on the order of the loosening of the stratum spongiosum of the endometrium during menstruation. This idea is certainly new. Whether or not it is normal is open to question. We are dealing here with a follicle which is (1) definitely small, (2) under the influence of a massive dose of gonadotropin, which was (3) applied near the time of normal ovulation. In this ovary the effective agent was certainly specific for this one follicle, since the recorded changes were not present in the two adjacent follicles, although one was just as near to the surface and the other was larger in size.

One more confusing factor is the presence near the rupture point of typical decidual cells (fig. 1*B*) in the form of an excrescence. It is very tempting to associate this with the rupture, but the position was doubtless purely incidental, since the excrescence could be traced 3 mm. distant. The cells were typical. They measured 30 microns. Their origin and function under the conditions are unknown. Not infrequently, however, decidua-like reactions of the serosa of the appendix have been observed during pregnancy. It is, therefore, quite probable that the decidual excrescence may have been part of a pregnancy-like response due to the administration of pregnant mares' serum (equine gonadotropin).

Lastly, one more upsetting feature comes into the picture. A luteinizing response was observed in other sites than the follicle under discussion (fig. 2*A*). A sudan III stain for fat in the section of the other ovary showed the presence of fat in (*a*) the granulosa cells of some of the maturing follicles and (*b*) in the luteinized theca cells of these follicles (fig. 2*B*) in a state of development comparable to that of the ruptured follicle. Since many sites gave this reaction, it is probable that the administered gonadotropin played an important part in stimulation. One of the most conspicuous of these was among the cells of an old corpus albicans. Here the particles were many and granular within a cell rather than spheroid and coalescent, suggesting phagocytosis rather than a metabolite. Another site for the fat reaction was in the theca cells of small secondary follicles. We also found the stain in the granulosa cells of the primary follicles and in the cytoplasm of such follicles.

EXPLANATION OF FIGURE 1

A, ovaries of a woman aged 22 who was submitted to laparotomy on ninth day of the menstrual cycle following a course of gonadotropin medication. Note the raised area on the left ovary (*a*) and the multiple small cystic areas in the right ovary (*b*). *B*, histologic section through the raised area in the left ovary (hematoxylin-eosin stain; × 65). Note the interstitial extravasation, the stratum granulosum, the hypertrophic theca interna cells, the decidua-like excrescence and the follicles.

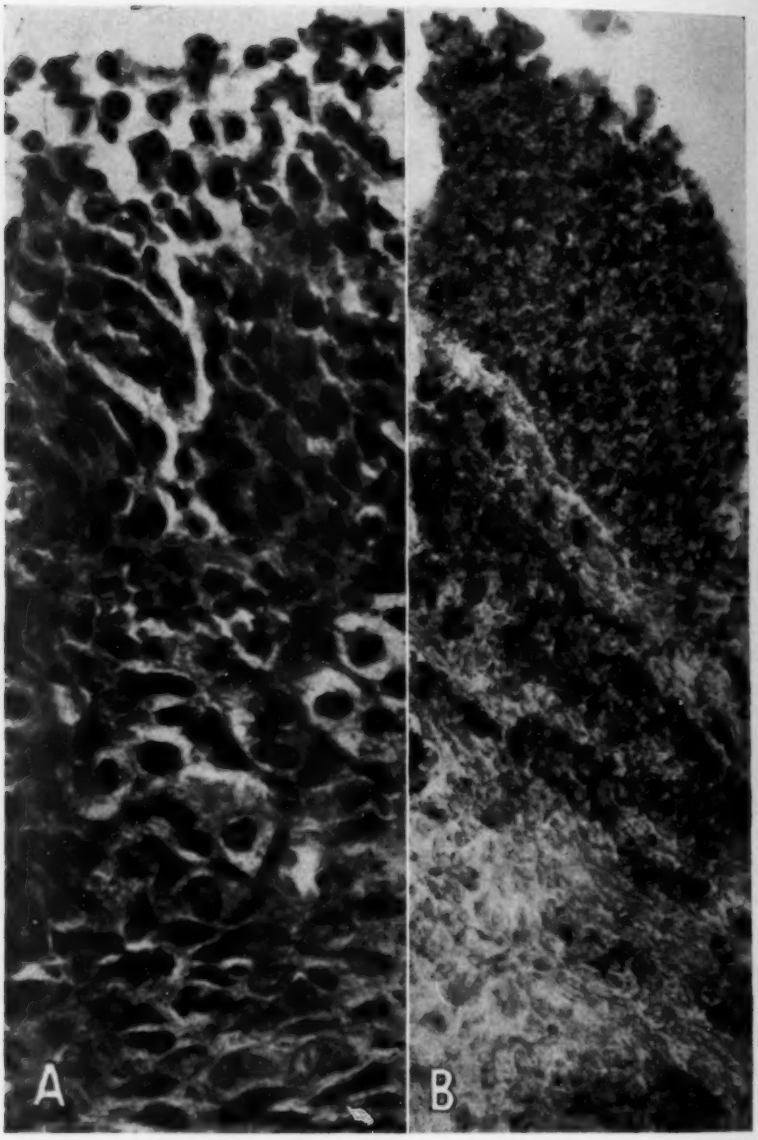

Fig. 2.—*A*, histologic section of the biopsy specimen from the right ovary (hematoxylin-eosin stain; × 600). Note the streaming of the granulosa cells, the occasional mitotic figures, the congested vessels and the hypertrophy and vacuolation of the cells of the theca interna. *B*, histologic section of the biopsy specimen from the right ovary through a maturing follicle (sudan III stain; × 250). Note the lipoid deposition (photographs black) in the cells of the stratum granulosum and particularly the cells of the theca interna.

SUMMARY

The picture here presented of an ovum about to be washed out of a ruptured maturing follicle is in keeping with that described for the various early corpora lutea by Allen and co-workers. It includes collapse, bloody gel, fibroblast invasion and granulosa cell hypertrophy. The hyperemia and the extravasation are the points of variation, which

Fig. 3.—Hematoxylin-eosin stain; × 200. Note an apparently healthy ovum about to be washed out from the partially collapsed follicle on the ninth day of the menstrual cycle following a course of treatment with equine gonadotropin. Note the dehiscence of the capsular part of the ovary; also the rupture point, the basophilic coagulum, the ovum still situated in a recognizable cumulus, the decidua-like excrescence and the Call-Exner body.

confuse rather than simplify the picture and lead to one more theory of ovulation. These changes may be an overemphasis of the normal brought about in a follicle not quite ripe by the injection of massive doses of gonadotropin three to five days before expected ovulation.

General Reviews

TATTOOS

A SURVEY, WITH SPECIAL REFERENCE TO TATTOOS AND SCARS AS INDICATORS OF SYPHILIS

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The tattoo is a widespread form of body marking which has persisted through the ages in spite of its proscription by tribal tabu, royal edict and biblical admonition. It preserves features of body painting and scarification, which are still practiced separately, and both of which are major steps in its evolution. The word "tattoo" is Polynesian in origin, and the most complete treatises on the subject are based on the customs of Oceania. Hambly [1] expressed the conviction that tattooing among primitive peoples has a significant religious background, because primitive man approaches nonhuman forces by positive rites, carried out with minute accuracy, while at the same time employing a number of negative rites or taboos. The association of such ceremonials with body marking is an indication of the importance of tattoos. This is especially true when, in addition to caution, secrecy and ritual, steadfast beliefs exist relating to the value of tattoo marks in heaven, dedication to a deity or limitation of the tattooer's craft to priests. Some forms of body marking related to magic and religion are difficult to define. The barrier of language encountered by investigators and, above all, the reluctance among natives to impart information about magicoreligious observances complicate the problem of untangling the real significance of specific marks. To befog the investigator further, the names and meanings of tattoo patterns are frequently unknown to the natives themselves. Barton [2] commented on the imperfect knowledge of patterns possessed by the native male. He obtained most of his information from the women natives of New Guinea. They are the tattooers and the tattooed in that locality, the marks being optional with men except with those who have taken human life.

From the Norman Bridge Pathological Laboratory, Rush Medical College of the University of Chicago.

1. Hambly, W. D.: The History of Tattooing and Its Significance, London, H. F. & G. Witherby, 1925.

2. Barton, F. R.: J. Roy. Anthrop. Inst. **48**:22, 1918.

Polynesian legend credits the poor memory of a messenger for tattoo marks on male Tongans. A Tongan emissary who had been sent to Fiji to observe the art and ritual of tattooing observed that only the women were tattooed. On his voyage home he repeated constantly, "The women must be tattooed but not the men." All went well until he reached his native shores. Then, according to Ploss, Bartels and Bartels,[3] he fell while running to his home, and forthwith forgot the formula. When he recovered from his stunned condition, he proclaimed, "One must tattoo the men but not the women"! The men of this tribe have been tattooed since then.

The employment of tattoos for identification is universal. Members of wild tribes find tattoos convenient for recognizing friends and enemies on jungle trails and in battle. Deeds of prowess recorded in skin script help decide the social order for a warrior and permit members of his family to receive commensurate adornment. In addition, the proscription of mating by persons with the same tribal markings fosters exogamy.

The Sioux warriors tattooed their faces for identification on the road to heaven. The path was presided over by an old woman whose critical inspection demanded such marks. Lacking them, the warrior was pushed from a cloud to become an aimless earthly wanderer, with no chance to enjoy the hereafter in the land of many lodges.

Certain tribes of Oceania substitute a form of necklace for the tattoo. Many others confine tattooing to women, so the latter may be identified in heaven by their husbands. Again, the Ekoi tribes employ scarification, with resulting round keloid formation, to provide funds for the purchase of food in the other world. When the spirit is hungry, it peels off a skin coin and satisfies its needs.

The relationship of tattoo to sexual development is unmistakable. The Long Glat women are tattooed at the onset of the menses and subsequently have designs placed on the hands, feet and, eventually, thighs. The completely tattooed woman, according to Hambly,[1] may bathe in the heavenly river and gather pearls; the partially marked may sit on the bank; the untattooed is not allowed near the river. Barton [2] described the ceremonial preceding the tattooing of Waima girls. They wear ornaments borrowed from members of their clan and display themselves in their finery in the village. The tattooed girls of nubile age formerly had been displayed on a platform after their tattoos were completed. The tattooing is done by women; the tattooer traces a pattern on the patient's skin with a palm leaf rib dipped in tree gum soot and drives in the carbon by a thorn tapped with a wooden striker. The

3. Ploss, H.; Bartels, M., and Bartels, P.: Woman: An Historical, Gynecological and Anthropological Compendium, St. Louis, C. V. Mosby Company, 1936.

thorn is a popular tattooing instrument, the use of which is associated in legends in various ways. Low caste Hindus fear their parents will not recognize them in heaven unless they are tattooed. In addition, they may incur the wrath of the God Parameshwar, who may cause a woman to be reborn as an evil spirit. The god at times, however, is lenient and allows her to enter heaven after she has been dragged through thorns, which produce the necessary markings.

The tattoos of a purely magical nature often are associated with attempts to ward off disease and accident. In some South American hill Indians, tattoos are imprinted about the eyes to guard against the evil eye. Similarly, the mouth is protected in many tribes. The vaginal orifice is surrounded frequently by elaborate designs. Not only the labia are ornamented but vertical stripes and intricate patterns are used on the abdomen. In Oceania, Barton [2] noted a common association of feathers and hair, especially pubic hair. The words for feathers and hair, in fact, often are identical. In women, pubic and axillary tattoos are the rule. The thighs and nates also are decorated in a profuse fashion. The nostrils are provided with imaginary barriers carried on the nose and cheeks, and kept safe by the insertion of nose sticks. Ornaments on ear lobes with or without tattooing serve the same purpose.

Tattoos depicting ferocious animals apparently are designed to terrify the enemy in battle. The tiger principally is used for this purpose. In some tribes it may be used as a matter of choice, but in others the actual killing of a tiger is a prerequisite for the display. Where social standards permit individual patterns in addition to the minimum markings of the group, animal designs are common. They are chosen to impart to the wearer the cunning, strength, speed or longevity of the depicted animal. Fish depiction is rare, but large soaring birds often are illustrated. There are routine marks for the frigate bird and hornbill in practically all South Pacific islands.

Special markings for members of secret societies are displayed usually on the forehead or another part of the face where they may induce the proper respect in the uninitiated. Occasionally the members of such societies are motivated by high ideals and curb and punish antisocial persons of the clan. More often the societies are a force of evil, parasitic on the community and guilty of the most heinous crimes. They have had their counterparts in all early centers of migration. Among the most powerful secret gatherings of this class is the Duk Duk.

The correlation of tattoo patterns in widely separated parts of the world has led most ethnologists to conclude that a common basis of thought and emotion exists in human beings. Modifications of this basis occur in the centers of population and culture established by migratory waves. The original tattoo designs are altered to include

the familiar objects of the new environment. The Eskimo, for instance, substituted polar bear designs for those of the hornbill. In many instances, only the arrangement of patterns and the time of application coincided with those of the ancestors before the dispersal of emigrants. The elaboration of the art of tattoo in the remote centers of communal life rendered the recognition of the archetype difficult. The crude, often self-inflicted marks required of individuals to conform to the social unit were supplanted by a geometric stage.[1] This reached its acme of perfection in the Moko of New Zealand, where a high degree of individuality of design was evolved, so much so that in 1815, purchases of land from New Zealand chiefs were legalized by a copy of the Moko of the seller on the deed. Later, when man recognized the fundamental principles of color, form and perspective, an opportunity for elaboration, animation and detail arose. The darkest skins being unsuitable for tattooing, their owners resorted to scarification and body painting.

The technic for tattoo has varied with the materials available for the process and the ingenuity of the tattooers. The simplest performance consisted of outlining the design on the skin in soot, with the finger or a twig, then driving the black particles into the skin by a thorn or a stone struck with a stone or a club. Broad lines were obtained by employing hoelike devices, called bodkins, many of which have been found in Egyptian tombs with strikers and tattoo pigments. In a later stage, one to eight needles, bound together and inserted in a handle, replaced the thorn. Elaborately carved ivory or gold strikers were fashioned, and the pigments were increased. Although many of these were sufficiently smeary to cling to the skin during the process of skin puncture, the favorite solvents were urine and saliva. Even the electric needle has not removed entirely the dangers attending the use of saliva, for pigment sometimes clings to the needle bundles and is sucked out by the operator. Saliva is used also to wash away the blood from the puncture so that the field of operation may be kept clear. Saliva enriched with tobacco juice is considered efficacious in relieving the pain after tattoo and in hastening recovery.

The colors achieved in tattooing are to a great extent standardized. Though gunpowder may be used, india or china ink is employed mainly in the blue portions of tattoos. The red effect is commonly due to particles of cinnabar (mercuric sulfide) pricked into the skin. Kurkuma is used alone for yellow and combined with indigo for green. Variations in the pigments are obtained principally by substituting less durable carmine for cinnabar and lamp black for ink. In tattoos on civilized people, blue predominates. When a second color is added, it usually is red. The simultaneous employment of blue and red has been followed repeatedly by reactions limited to the skin in the vicinity of one or the other.

Blue zones react to a variety of stimuli, indicating hypersensitivity. Brose [4] spent a year and a half studying reactions in tattoos subjected to mechanical, actinic and chemical stimuli. After ten blows on the tattooed parts the skin became swollen in the blue portions in fifteen minutes and the tattoo became more distinct. The reaction disappeared in thirty minutes without having produced changes in the red designs. When the tattoo was covered with a blue cloth and rubbed, the blue parts became swollen but the red reacted only slightly. The reactions in the colors were reversed when a red cloth was employed. Results similar to those obtained with cloths were obtained with red or blue arc lamps. With lamps, reflexive reactions also could be elicited. For example, if the normal upper arm was subjected to irradiation, the tattoo on the forearm would react as if the stimulus had been applied directly to it. The reactions to all stimuli varied with the site involved. They were strongest on the flexor surfaces of the arms and less intense on the extensor parts of the arms, the back, the chest, the legs and the face. With repeated stimuli, the reactions weakened everywhere. Hot water applied for fifteen minutes achieved the same results as other stimuli. In all tattoos over three years old, only the blue parts responded. The explanation for this peculiar behavior of the skin was sought in damage to the lymphatic and vasomotor systems by the foreign particles of the tattoo.

The predilection of secondary and tertiary cutaneous syphilids for the blue portions of tattoos is significant. Dohi [5] reported such localization in a man 35 years old who had been tattooed when 19 years old. Although there were eruptions on the untattooed parts of the body, the heaviest papular clusters were in the darkest part of the tattoo. The red places were spared. Holland [6] saw a similar localization in the tattoos of 3 men. One of these tattoos was faded, but even in this the secondary papular syphilids were evident. In a fourth man he found a secondary eruption in the blue and red figures, more marked in the blue. This patient had an unusually extensive dermal rash, which spared only the hands and feet. Florange [7] reported a papular syphilitic eruption in the blue parts of a tattoo while the red parts were uninvolved. He also, however, noted that the syphilitic eruption of another patient localized in the red marks. He believed the places most stimulated were those which reacted to the syphiloderm.

Additional reports of the strict localization of syphilitic eruptions in tattoos invariably stress the blue involvement. Belote [8] concluded that

4. Brose: Dermat. Wchnschr. **84**:46, 1927.
5. Dohi, S.: Arch. f. Dermat. u. Syph. **96**:3, 1909.
6. Holland, W.: Arch. f. Dermat. u. Syph. **110**:393, 1911.
7. Florange, A.: Dermat. Ztschr. **16**:783, 1909.
8. Belote, G. H.: Arch. Dermat. & Syph. **18**:200, 1928.

red pigment never had been proved to be cinnabar in those cases in which secondary syphilitic papules were found in it. In 2 men he observed an apparent barrier in the red zones. Zechmeister [9] and Wechselman [10] each observed 2 patients in whom secondary syphilids followed the contours of tattoos. In one, the eruption occurred in the remnants of a partially removed tattoo.

The particles of foreign material in the skin in tattooed areas have been regarded as a locus minoris resistentiae or as an irritant just as are many chemical and mechanical stimuli which induce secondary syphilitic eruptions of the skin. The well known examples are the corona veneris which appears on the forehead under a tight hat band, the stimulus supplied by scratching the skin, especially where the clothing is louse infested, and the luxuriant syphilitic infiltration wherever maceration occurs from perspiration, sebum, mucus or pus. Tarnowsky [11] sought to simulate maceration by the use of powerful salves. The hyperemia and ulceration which he produced in the skin induced syphilitic eruptions, most easily in syphilis in the acute condylomatous stage and least effectively in syphilis of the bones and joints. Although he failed to provide a means for identifying systemic syphilis in the days before the Wassermann test, his experiments created unusual interest in the delayed cutaneous manifestations of syphilis.

The "cauterisatio provocatoria" employed by Tarnowsky [11] to distinguish between healed and active syphilis also produced infiltrates around the cauterized areas in persons with carcinoma, caseous pneumonia, arteriosclerosis, pruritus senilis and psoriasis vulgaris. Other investigators did not agree with him. Foremost among the dissenters were Kaposi,[12] Köbner [13] and Rinecker.[14] There was confirmation of the work of Tarnowsky [11] by Neumann,[15] who regarded the cauterization as dangerous and likely to stimulate quiescent syphilis into activity.

The red portions of most tattoos are made with mercuric sulfide, which has been credited repeatedly with antisyphilitic properties. It is more irritating than the other materials forced into the skin and may cause itching periodically for as long as three (Dohi [5]) and even seven years.[16] At times, nodules have persisted in the skin around the red

9. Zechmeister, H.: Monatsh. f. prakt. Dermat. **32**:225, 1901.

10. Wechselman, H.: Dermat. Ztschr. **12**:557, 1905.

11. Tarnowsky, B.: Vrtljschrft. f. Dermat. **4**:19, 1877.

12. Kaposi, M.: Pathologie und Therapie der Hautkrankheiten in Vorlesungen für praktische Aertzte und Studirende, Vienna, Urban & Schwarzenberg, 1880.

13. Köbner: Vrtljschrft. f. Dermat. **5**:589, 1878.

14. Rinecker, cited by Neumann.[15]

15. Neumann, I.: Syphilis, in Nothnagel, H.: Specielle Pathologie und Therapie, Vienna, Alfred Hölder, 1896.

16. Madden, J. F.: Arch. Dermat. & Syph. **40**:256, 1939.

deposits. Histologic examinations have shown these to be severe chronic inflammatory reactions with slight hyperkeratosis, parakeratosis and moderate acanthosis in the epidermis. The infiltrate is most dense about the particles of pigment in the lower part of the corium and consists of lymphocytes, eosinophils and fibroblasts. The nodules are not the result of traumatization, for the red and blue parts of tattoos are produced by the same technic. Cutaneous patch tests with various mercury compounds have disclosed that some patients have marked sensitivity to mercury. Arning [17] studied such a patient in detail and saw 3 others. Unna [18] and Ballin [19] also noted mercuric dermatitis in tattoos after the employment of mercury compounds elsewhere on the body. The reaction may be so severe that a keloid-like tumor develops about the mercuric sulfide. Ullmann [20] reported such an occurrence in 1903: the protruding skin nodules were excised and revealed chronic inflammation about the particles of the mercury compound in the tattoo. Two such growths were reported in 1938 by Müller [21] and Schmitz.[22]

A usual course of events follows tattoos. A marked irritation is produced, and the skin becomes swollen and hot. The design is hidden by seepage of serosanguineous material, which forms a crust. After seven to eighteen days, the crust falls off, and with the bridging of the puncture holes by epithelium, the design becomes clear. It is not unusual for the red parts which have been executed with mercuric sulfide to remain swollen for several weeks and to itch even after the swelling has subsided. The advent of the electric needle has shortened the postoperative course. Cold water, saliva, urine or whisky is used to wash the crusted region. Urine and saliva are used most frequently by itinerant tattooers because of their availability.

The danger attending the use of saliva was described first by Hutin,[23] in 1853. He reported the transmission of syphilis from the artist to his patient by the saliva used to mix the dyes. Josias [24] saw three chancres in the tattoo of a man 19 years old, about two months after the operation. Typical secondary manifestations occurred a month later. Eight companions of the unfortunate patient were infected by the same operator, who was being treated for active syphilis and had mucous patches in his mouth at the time. Such multiple chancres as were noted

17. Arning, E.: Arch. f. Dermat. u. Syph. **123**:225, 1916.
18. Unna, P. G.: Arch. f. Dermat. u. Syph. **160**:153, 1930.
19. Ballin, D. B.: Arch. Dermat. & Syph. **27**:292, 1933.
20. Ullmann, J.: Monatsh. f. prakt. Dermat. **37**:49, 1903.
21. Müller, O.: Dermat. Wchnschr. **106**:6, 1938.
22. Schmitz, H. J.: Dermat. Wchnschr. **107**:1404, 1938.
23. Hutin, E.: Bull. Acad. de méd., Paris **18**:348, 1853.
24. Josias, A.: Progrès méd. **5**:205, 1877.

by Josias were encountered also by Cheinisse,[25] who found five of varying size and development in the tattoo of a blacksmith. The outstanding American investigation of the transmission of syphilis by infected saliva was that by Maury and Dulles,[26] published in 1878. They treated a group of men who had been tattooed by a vagrant who had contracted syphilis in February 1877. He had large mucous patches in his mouth in April and May while he was practicing his art on numerous men on a circuit from Philadelphia to Reading and from Jersey City to New York. His designs were handsome and cost from a drink of whisky to $2. Of 22 patients examined by Maury, 4 had never had syphilis yet were not inoculated, although the artist had used his saliva to mix the colors; 3 had had syphilis before being tattooed, and 15 never had had syphilis but acquired it by tattoo. Whitehead [27] observed a similar outbreak in the Indian army, in which a dozen men were tattooed by an artist-private who had mucous patches in his mouth. Although the operator used the same needles on all the men and cleaned their skin surfaces with saliva, only 5 contracted syphilis. Each had a solitary indurated chancre, which occurred at the first point where the needles had been inserted.

Barker [28] studied an outbreak of syphilis in the British army. Twelve men in one regiment acquired syphilis from a hawker in the barracks who tattooed as a sideline. In his mouth were mucous patches, yet he used saliva to mix his colors and to wipe away the blood resulting from the needle pricks. Several of his patients had two hunterian chancres in their tattoos; others had as many as four rupial ulcers. The incubation period was from thirteen to eighty-seven days, and ulcers or chancres appeared only on one arm even though in several instances both arms had been tattooed.

In investigating the frequency of secondary syphilitic contagion among enlisted men in the United States Army and Navy, Arthur [29] discovered some interesting facts about the transmission of syphilis by tattoo. The average number of men under observation in a ten year period was 33,760. The surgeons collaborating in the study had an average of eighteen and a half years' experience and were familiar with syphilitic infections. They reported only 41 cases of extragenital primary lesions, in 26 of which the lesion was the result of tattooing by a man who moistened needles with his saliva. Mucous patches were found in the mouth of this tattooer by Capt. T. E. Wilcox.

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25. Cheinisse, L.: Ann. de dermat. et syph. **6**:15, 1895.
26. Maury, F. F., and Dulles, C. W.: Am. J. M. Sc. **75**:44, 1878.
27. Whitehead, H. R.: Brit. M. J. **2**:601, 1889.
28. Barker, F. R.: Brit. M. J. **1**:985, 1889.
29. Arthur, G.: M. Rec. **30**:674, 1886.

The potentialities for transmitting other forms of contagion in saliva are numerous. Bercheron [30] in 1862 reported 43 cases of infection, in which 8 deaths occurred as a direct consequence of tattooing. In 8 cases, amputation was necessary; in 7, gangrene was present. The disclosures of Bercheron were in great measure responsible for a French governmental decree forbidding the tattooing of enlisted men. In addition to the frankly septic infections following tattoo, those of tetanus, leprosy and tuberculosis have been cited by Shie.[31] Some cases of tuberculosis, such as those reported by Dore,[32] are questionable, as even the authors believed reservations necessary in their reports. Ullmann,[20] on the other hand, cited the well authenticated cases observed by Collings and Murray.[33] A 15 year old boy suffering from pulmonary tuberculosis tattooed his 2 brothers and a friend and rubbed the wounds with his saliva. Shortly afterward the tattooer died and tuberculous pustules developed in the tattoos of his subjects, and lesions in their cubital and axillary lymph glands. Heller [34] reported the development of tuberculosis in a tattoo which had been rubbed with infected cow's milk.

The permanence of tattoos has intrigued many physicians. The durability of these cutaneous designs depends in general on the pigment employed, the method of insertion and the physiologic changes in the skin. Blue or black pigments are the most permanent, and bodkins produce the most lasting impregnation. The electric needle produces the least durable patterns, hence necessitates periodic refreshing of the colors, thereby exposing the patient to infection each time the operation is repeated. Professionally tattooed persons who act as dime museum or circus exhibits have to be redecorated in their yellow, green and red figures about every ten years.

The fading of tattoos is a variable process which has led to errors in identification. Casper [35] is credited with the first report of a medicolegal nature involving a faded tattoo. The case concerned a murdered man whose tattoo had been seen by two physicians respectively nine and three years before his death. No tattoo was visible at autopsy or at three subsequent exhumations. It was decided finally that the tattoo had faded. In arriving at this conclusion, Casper studied the tattoos of 36 men in an invalids' home. He found that four tattoos had disappeared completely, two had faded partially, three had faded badly and one was exceptionally well preserved after fifty-four years.

30. Bercheron, E.: Union méd. de la Gironde **6**:225, 1861.
31. Shie, M. D.: J. A. M. A. **90**:94, 1928.
32. Dore, S. E.: Brit. J. Dermat. **25**:257, 1913.
33. Collings and Murray, cited by Ullmann.[20]
34. Heller, cited by Ullmann.[20]
35. Casper, J. L.: Vrtljschr. f. gerichtl. u. öff. Med. **1**:274, 1852.

The modern tattoo artists shave the skin of their clients and cleanse it with soap and water and alcohol. The needles are electrical devices run by vibrator coils. With fair aseptic precautions, the opportunity for the contraction of syphilis is remote. However, an uncleaned needle used first on a syphilitic, then on a healthy person could cause the same tragedy as with the older method.

The motives underlying modern tattooing are apparently as complex as those of savages. Psychologists are in some measure in accord on certain aspects of tattooing. The herd motive is evident in teen-aged boys who go in a group to the "professor's" shop to be tattooed. The boy who refuses to submit to the process because of the pain is an outcast. Parry [36] cited widespread tattooing in New York children during a certain school vacation. Sex plays a major role in the tattoo tendency of men and women. Men in the navy, mines and isolated places have been in the past the main clientele of tattooers. Lacking the physical companionship of women, they resort to the images of women on their skins. Their sexual appetites are, at times, partly gratified by tattoo artists who use their knowledge of anatomy in applying figures so that muscle activity ripples the skin and animates the figures. The sublimation of a sexual act into observation of tattoo patterns is thus repeatedly resorted to.

In women, tattoos are found most often on prostitutes. On some, as many as thirty names of lovers have been inscribed on the breasts and abdomen. The main problem is the altering of the lover's initials when his love wanes. Some of the prostitutes, of course, are the victims of salesmanship. Bergh,[37] in his study of tattoos among 804 prostitutes of Copenhagen, Denmark, found 80 adorned with such markings. The work on 49 was done by one artist who had only recently set up shop. The rest of the women had been tattooed by their lovers or women friends. In 34, only initials were used; in 10, names plus initials; in 22, initials and figures; in 11, names and figures; in 3, figures alone. The inscription "For Ever," hearts, and hearts pierced by daggers were the favorite designs.

There are aspects of totemism in the weakling who has his arm emblazoned with the same patterns which grace that of his hero. The magic strength imparted to the jungle man by his tiger tattoo exerts its subtle influence through the ages. Less complimentary is the interpretation of tattoos on criminals by Lombroso.[38] He expressed the opinion that modern tattoos represent atavistic reversions to primitive

36. Parry, A.: Tattoo: Secrets of a Strange Art as Practiced Among the Natives of the United States, New York, Simon & Schuster, Inc., 1933.

37. Bergh, R.: Monatsh. f. prakt. Dermat. **12**:205, 1891.

38. Boselli, A., and Lombroso, C.: Arch. di psichiat. **8**:1, 1887.

criminal types. Such an interpretation is too harsh to be applied to civilized, noncriminal persons. The sadistic and masochistic elements of tattoo are more apparent.

The tendency to be tattooed as a pastime is evident in the reasons offered by many persons who have tattoos on their bodies. Perhaps this attitude is best summarized in a quotation by Parry. "The origin of tattooing is doubtless the same as the origin of whittling—pure brainless indolence. But while the civilized Yankee merely whittles at his chair, or a piece of soft wood, the untutored and childish savage naturally preferred whittling at his fellow creatures." Whatever the rational explanation for tattoos, a deeper motive seems discernible in their acquisition by most persons. The vast number of publications dealing with theories explaining these motives is proof of a psychologic and ethnologic interest. Tribal studies are likely to show a gradual diminution in occurrence. In civilized countries there are certain to be more opportunities for such studies because of the present huge mobilization of fighting men throughout the world.

Although numerous writers have stated that a tattooed person may be regarded as syphilitic, relatively few scientific efforts have been made to prove the relationship. Hamada [39] performed the Wassermann test on the blood of 265 tattooed iron workers at Yawata, Japan, and found 20 per cent with positive reactions. He further correlated the motif of the tattoo with the positiveness of the test and concluded that 21 per cent were tattooed from curiosity, 11 per cent because of illness, 33 per cent for sexual reasons and the rest for unclassified reasons.

Despite the lively interest in tattoos as possible sources of syphilitic infection, there is no accurate statistical study of the incidence of syphilis in the bodies of the tattooed dead. The conception that a tattooed body is likely to be syphilitic is as widespread as the assumption that a tattoo is an almost certain sign of a positive Wassermann reaction. The fallacy of this assumption is known to experienced pathologists and is proved readily by studies of large numbers of autopsies. The bodies inspected by coroners' physicians represent in any large community a reliable cross section of the inhabitants. Such a group was used in this study.

The records of the late Dr. E. R. LeCount during his incumbence as coroner's physician at the Cook County Hospital in Chicago form the basis for the following compilation. A histologic study of many of the cases has been carried on by me during the last ten years, although the autopsies were completed in August 1924. In the series, 9,510 bodies were deemed suitable for this review. Their classification by race and sex, as adults and children, is as follows: There were 6,582

39. Hamada, H.: Bull. Soc. japon. de syph. **13**:28, 1935.

white male and 1,267 white female adults; 383 white male and 268 white female children; 625 Negro male and 26 female Negro children; 13 adult male and 1 female Asiatic; 16 adult male Mexicans, and 1 white adult hermaphrodite.

The grouping was divided into tattooed nonsyphilitic, tattooed syphilitic and untattooed syphilitic persons (see table 1). Simultaneously, genital and cutaneous scars regarded as syphilitic were tabulated. Of the 6,582 white male adults, 579 were tattooed but nonsyphilitic, 474 were nontattooed but syphilitic and 130 were tattooed and syphilitic. A total of 709 white men, therefore, had submitted their bodies for ornamentation by tattoo. This is in sharp contrast to the adult white females, only 13 of whom, in a group of 1,267, were tattooed. Those

TABLE 1.—*Incidence of Tattoo Alone, of Syphilis Alone and of Tattoo and Syphilis*

	Total Bodies	Tattoo Alone		Syphilis Alone		Syphilis and Tattoo	
		No.	%	No.	%	No.	%
White persons							
Males	6,582	579	8.79	474	7.20	130	1.97
Females	1,267	9	0.71	39	3.07	4	0.31
Negroes							
Males	625	9	1.44	116	18.56	15	2.40
Females	275	5	1.81	23	8.34	0	0
Asiatics							
Males	13	2	15.2	1	7.61	0	0
Females	1	0	0	1	100	0	0
Mexicans							
Males	16	2	12.5	2	12.5	1	6.25
Females	0	0	0	0	0	0	0

tattooed numbered 9; those with syphilis and tattoo, 4; those untattooed but syphilitic 39. The number of men with tattoo alone among the 625 male Negro adults was 9; that of untattooed syphilitic men was 116, and that of tattooed syphilitic men, 15. Of the 13 male Asiatics, simple tattoos were found on 2 and syphilis in 1. There were no male Asiatics with tattoo and syphilis. The single Asiatic female was untattooed but syphilitic. Of the 16 Mexican males, 2 were untattooed, 2 were syphilitic and 1 was tattooed and syphilitic. The age incidence is interesting. Eleven white males between the ages of 10 and 20 years were tattooed. With regard to the other races, no marked bodies less than 20 years of age were found. For all races, the greatest number of tattooed bodies were between the ages of 30 and 50 years. Most of these bodies were those of white males between 40 and 50 years, i. e., 166 of the 579 noted at all ages.

A small group of males had tattoos and genital scars but no evidence of syphilis in their bodies. Of these, 10 were white and 5 were Negro males. The scars were located thus: 10 on the glans penis, 2 on the shaft of the penis and 3 on the foreskin. The distribution of chancre scars,

even in so small a group, proves the impracticability of tattooing the glans penis with mercuric sulfide as a prophylactic against syphilitic infection as has been suggested.

Tattooed bodies that were syphilitic were overwhelmingly those of white males. Two had scars resembling that of a chancre in a tattoo. The incidence of genital scars in these bodies was 20 per cent, and 1 body had a healing chancre. A man who had a penile scar and knew that he had had syphilis lost part of his genitals in a crushing injury shortly before death. The scar, therefore, was not visible post mortem. The incidence of syphilis and of scars of tattoo chancres and the loca-

TABLE 2.—*Distribution of Visceral Syphilis in Tattooed Syphilitic Persons: A Comparison with Cutaneous Lesions*

	White Persons		Negroes		Mexicans	
	Males	Females	Males	Females	Males	Females
	130	4	15	0	1	0
Syphilis of						
Aorta	44	2	9	..	1	..
Aneurysm	7	..	..	..	..	..
Liver	22	3	2	..	1	..
Testes	5	..	..	..	..	..
Leptomeninges	28	1	4	..	..	..
Bone	1	..	..	..	..	..
Bowel	..	..	1	..	..	..
Blood (positive Wassermann reaction)	22	1	..	..	..	..
Coronary arteries	..	..	1	..	..	..
Kidney	1	1	1	..	..	..
Spleen	1	..	..	..	..	..
Stomach	1	..	..	..	..	..
Cerebral arteries	1	..	..	..	..	..
Skin	45	1	4	..	..	..
Heart	1	..	..	..	..	..
Brain	3	..	..	..	..	..
Adrenal gland	1	..	..	..	..	..
Larynx	1	..	..	..	..	..
Spirochaeta pallida (in chancre)	1	..	..	..	..	..

tion of penile scars in this group were as follows: Twenty-six of the tattooed syphilitic white males had penile scars: 17 scars were on the glans, 4 on the foreskin and 5 on the shaft. Mucous patches were encountered in the mouth of 1 body. Chancre scars were seen in 2 tattoos, an incidence disproportionately low for a source long considered a main source of syphilitic contagion. In sharp contrast is the high incidence of scars in the skin, which were found in 45 men. No genital scars were found in the white or Negro female adults. Of the bodies of tattooed syphilitic Negro male adults, 7 had scars on the genitals, 6 on the glans and 1 on the shaft. In none of the tattoos were there scars resembling those left by chancres, but scars of syphilids were noted elsewhere in the skin in 4.

Table 2 presents the occurrence of syphilis in the individual organs of tattooed syphilitic persons, the incidence of positive Wassermann

reactions and the isolation of Spirochaeta pallida from a genital chancre. The total number of lesions exceeds the number of bodies because in many instances several organs were involved.

The importance of cutaneous scars as indicators of visceral syphilis is apparent from this tabulation. Such scars were present in 25 per cent or more of the bodies of white males and females and Negro males. In a group of 130 white males there were 45 with cutaneous scars, and 44 of these had syphilis of the aorta. This represented the closest agreement between cutaneous scars and the commonest visceral syphilitic lesion.

TABLE 3.—*Distribution of Lesions in Adult Syphilitic Persons Without Tattoo*

	White Persons				Colored Persons			
	Males		Females		Males		Females	
	No.	%	No.	%	No.	%	No.	%
Total	474		39		116		23	
Death from syphilis	87	18.0	13	33.3	32	27.5	9	39.1
Genital scar	99	20.0	0	....	57	49.1	0	....
Cutaneous scar	78	16.4	8	20.4	45	38.8	7	30.4
Syphilis of aorta	173	36.0	22	56.0	61	52.5	15	65.2
Cerebrosyphilis	115	24.1	16	41.1	27	23.2	4	17.3
Aneurysm of aorta	46	8.78	7	17.9	8	6.89	2	8.68
Ruptured aneurysm	18	3.79	0	....	10	8.62	0	....
Syphilis of liver	79	17.3	14	35.8	34	29.3	7	30.4
Syphilis of spleen	16	3.37	2	5.12	1	0.86	3	13.04
Syphilis of testes	25	5.27	0	....	5	4.31	0	....

TABLE 4.—*Distribution of Fatal Syphilitic Lesions in Syphilitic Adults with Tattoo*

	White Persons		Negroes	
	Males	Females	Males	Females
Death from syphilis	87	13	32	9
Syphilis of aorta	46	8	15	4
Cerebrospinal syphilis	22	4	4	3
Ruptured aneurysm	18	0	10	0
Syphilis of rectum with rupture	..	1	..	2

The syphilitic bodies without tattoos revealed syphilis as the direct cause of death in 18 per cent of the white males, 33.3 per cent of the white females, 27.5 per cent of the colored males and 39.1 per cent of the colored females. The distribution of the syphilitic involvement in the various organs is tabulated (table 3).

Table 4 presents a comparison of the four most common causes of death from syphilis in tattooed adult white persons and Negroes. Syphilis of the aorta was the main cause of death in all the cases. It was responsible for 52.8 per cent of the deaths in the white male adults and of 61.5 per cent of the deaths in the females. Among the Negroes the percentages were more nearly equal—46.8 per cent of the males

and 44.4 per cent of the females died from aortic syphilis. Cerebrospinal syphilis was twice as common in white males (25.1 per cent) as in Negroes (12.5 per cent). The difference between the percentage for white females (30.7 per cent) and that for Negro females (33.3 per cent) was slight. No female of either race died of a ruptured aneurysm of the aorta, but this dramatic death occurred in 20.6 per cent of white males and 31.2 per cent of colored males. Syphilis of the rectum with rupture was confined to the females of both races and accounted for 7.77 per cent of the deaths in white women and 22.2 per cent of the deaths in colored. In each instance it was accompanied by extensive peritonitis. The rupture always was associated with ulceration of the rectal mucosa 8 to 15 cm. from the anus and in the Negro women by heavy scar formation and rectal stricture. The site of these lesions was compatible with primary syphilitic inoculations in a region

TABLE 5.—*Distribution of Gumma in Syphilitic Persons Without Tattoo*

	White Persons		Negroes	
	Males	Females	Males	Females
Total with syphilis	474	39	116	23
Gumma of				
Heart	1	..	..	..
Pancreas	..	..	1	..
Liver	25	7	10	3
Leptomeninges	4	1	..	1
Kidney	2	..	1	1
Spleen	7	1	..	4
Brain	1	1	1	1
Testicle	1	..	..	..

where a chancre would be chronically infected or where trauma and repeated irritation would lead to the development of late syphilitic manifestations. Histologic sections from these lesions were studied carefully because of recent advances in the differential diagnosis of conditions such as venereal lymphogranuloma.

As the occurrence of gumma has constituted incontrovertible proof of syphilis, its presence was regarded as significant in this study. The largest groups available for comparison were the white and Negro male and female untattooed syphilitic adults. The results of the study, summarized in table 5, disclose some interesting contrasts. The women of both races had consistently greater percentages of gumma in their organs than the men. In the latter, however, a greater number of organs were involved. Gumma of the liver was the most common, except in Negresses, in whom 17.36 per cent of the gummas occurred in the spleen, compared with 13.02 per cent in the liver. The second site of predilection for gumma was the spleen; the third, the leptomeninges; and the fourth, the brain. Gumma occurred once each in the testicle, the myocardium and the pancreas.

SUMMARY

The psychologic bases of tattoo seem essentially the same in primitive and modern man, as also the choice of designs. The latter consist mostly of identification marks, ornamental figures and erotic or religious symbols.

Notwithstanding the modernization of the equipment used, unclean personal habits of the operator still jeopardize the health of his client. Syphilis, however, contrary to general opinion, is not commonly distributed by tattoo.

Pigments employed in tattoos frequently act as irritants in the corium, and the mercuric sulfide occasionally induces a local hypertrophy of the skin resembling a keloid. The slow absorption of mercury from mercuric sulfide also may sensitize the subject to this element so that the subsequent employment of mercurial drugs produces an allergic rash.

A study of tattoos and syphilis was made, based on the postmortem records and histologic preparations of 9,510 bodies.

Tattoos were encountered, in order of frequency, on male Mexican adults, male Asiatic adults, male white adults and male Negro adults. Although relatively few Negroes were tattooed, the percentages for the sexes in this race are in close agreement. As to white persons, 8.79 per cent of the males, but only 0.71 per cent of the females, were tattooed.

Penile scars and scars of cutaneous syphilids were more accurate indexes of visceral syphilis than tattoos.

Notes and News

Appointments.—Francis G. Blake, Sterling professor of medicine, Yale University, has been appointed dean of the Yale School of Medicine.

William T. Salter, research fellow in biochemistry of the Harvard Cancer Commission and assistant professor of medicine in Harvard Medical School, has been appointed professor of pharmacology at Yale University.

Society News.—The Minnesota Society for the Control of Cancer has been organized with Dr. William A. O'Brien, St. Paul, as president and Mrs. S. L. Carr, Minneapolis, as secretary.

American Journal of Cancer.—This journal will cease publication with the issue of volume 40, which will contain an author-subject index of volumns 15 to 40, inclusive.

Awards.—The 1941 Willard Gibbs Medal of the Chicago section of the American Chemical Society has been awarded to Edward A. Doisy, professor of biochemistry in St. Louis University, for his work on hormones and vitamins.

A bronze bust of Ernest E. Tyzzer, professor of comparative pathology and of tropical medicine in the Harvard Medical School, has been presented for placement in his department.

The first award of the Friedenwald Medal of the American Gastroenterological Association has been made. Walter B. Cannon, professor of physiology in the Harvard Medical School, has received the medal "in recognition of his pioneer utilization of the x-rays in gastroenterology, and his important contributions to the mechanics of digestion, to the elucidation of the sensations of hunger and thirst, and to the development of the science and practice of gastroenterology."

Obituaries

SIR FREDERICK GRANT BANTING, M.D.
1891-1941

From time to time there comes news that tells of some irreparable loss to the world, loss of a great worker of great deeds done, loss of great good that might yet have been done. Such was the news of the death of Sir Frederick Grant Banting, co-discoverer of insulin, killed in an airplane crash near Musgrave Harbor on the east coast of Newfoundland on Feb. 21, 1941, while on a military mission to England. He was one of the first of our great men of medicine to meet death in this Second World War.

Dr. Banting was born in Alliston, Ontario, Canada, on Nov. 14, 1891, the son of William Thompson and Margaret Grant Banting. He received his medical education in Victoria College, Coburg, Ontario, and the University of Toronto Faculty of Medicine, graduating with the degree of M.B. in 1916, and later receiving the degrees of M.D. (1922) and D.Sc. (1923). He was also to receive the honorary degree of LL.D. from Queen's University, Kingston, Ontario, in 1923, LL.D. from the University of Western Ontario, London, Ontario, in 1924, Sc.D. from Yale University in 1924 and D.Sc. from the University of the State of New York in 1931.

For three years he served with the Canadian Army Medical Corps during the World War, in which he was seriously wounded, and in 1918 he was awarded the Military Cross. On his return to Canada, he became resident surgeon of the Hospital for Sick Children in Toronto in 1919-1920, and during 1920-1921 held the appointment of part time assistant in the department of physiology of the University of Western Ontario. It was at this point, in November 1920, that he read an article by Barron on the surgery of the pancreas that led to a correlation in his mind of the pancreas and the problem of diabetes. Feeling that the secret of the hormonal deficiency in diabetes was to be found in the pancreas, he turned to Dr. J. J. R. MacLeod, professor of physiology of his alma mater, for technical advice and means to conduct his experimentations. This was extended to him by his assignment to the department of physiology in May 1921, where he started work in association with a research fellow, Dr. Charles Best, who now holds the chair of professor and head of the department of physiology. It was known that extirpation of the pancreas was followed by ascending blood sugar levels and early

death. It was then found that ligation of the pancreatic ducts led to almost complete atrophy and destruction of the pancreas, but the blood sugar levels remained unaltered and the experimental animal's health was not markedly affected. Microscopic examination of the pancreatic remnants indicated the persistence of glandular structures, unconnected with the external secretory zymogenous glandular system of the pancreas, and known as the islands of Langerhans. All of this was embodied as his basic idea in a three sentence notation in his notebook: "Tie off pancreatic duct of dogs. Wait six to eight weeks for degeneration. Remove residue and extract." Then commenced an arduous and almost heart-breaking task of extraction and isolation of the secretion of these islands, first from a series of such atrophied glands and finally from whole pancreases secured in mass from abattoirs. Ten dogs had been requested for these experiments, but more and more were found to be needed. At last, with help from Dr. J. B. Collip, a product (which later underwent further refinements, concentration and finally crystallization) was found which would maintain the life in a depancreatized experimental animal and which could destroy an animal by causing a drop of the blood sugar level in what is now recognized as insulin shock. Dog 92 was the animal which, previously made diabetic, "miraculously" showed prompt recovery from a dying condition on subcutaneous administration of the pancreatic extract. This was the effect and result which Dr. Banting and his colleagues had been striving for so persistently. Dr. Joseph Gilchrist, a classmate of Dr. Banting's, became one of the first of the millions of human beings to be released from the stranglehold of diabetes. This internal secretion product was then standardized on the basis of allowing the utilization by oxidation of a set amount of dextrose in the experimental animal, 1 unit of insulin being the amount necessary to lower the blood sugar of a 1 Kg. rabbit, fasting twenty hours, to 0.045 per cent. In 1921, the announcement was made of the discovery of a hormone of the pancreas which controlled the blood sugar level and would serve in the specific amelioration of diabetes. This hormone was ultimately named insulin in recognition of its origin in the islands of Langerhans of the pancreas. I can well remember, as one who was a medical student at the University, the tiny brick building, now disappeared, in which the production of insulin for medical use was started. The process of manufacture of insulin and its subsequent refinements were patented and placed in the name and trusteeship of the University of Toronto, setting the standard for the control of medical therapeutic products that is now followed by many universities and other institutions dedicated to public service. Since then, insulin has been manufactured by responsible firms throughout the world on license from the University of Toronto, which has maintained a constant check of standards on the manufactured insulins. By these means, such products are

made available to all at a reasonable cost, avoiding a possible prohibitive destructive monopolistic control, while the proceeds of such licensure have paid for and made possible other and further experimental work. Dr. Charles H. Best has been in charge of the production and control of insulin in the Connaught Laboratories of the University of Toronto since 1922.

Dr. Banting, during his primary work on insulin in 1921-1922, served as lecturer in pharmacology; later he became senior demonstrator in medicine, in 1922-1923, and at the same time received the appointment of honorary consulting physician to the Toronto General and the Toronto Western Hospital. Announcement was made in 1923 of the joint award in medicine of the Nobel Prize to Dr. Banting and Dr. MacLeod for their discovery of insulin. This they divided with Drs. Best and Collip in view of the intimate connection of the latter men with the research work on insulin. Subsequently in 1923, Dr. Banting was given the newly founded Professor Banting and Best Chair of Medical Research at his alma mater. The same year, a grateful nation granted him a life annuity of $7,500 by Act of Parliament of Canada. This was greatly appreciated, for outside of his Nobel Prize award Dr. Banting never received any financial return from the licensing or sale of insulin.

Recognition of the great importance of the discovery of insulin was prompt and world wide. Among the awards extended to Dr. Banting were: the Starr Gold Medal, in 1922; the George Armstrong Peters Prize, in 1922; the Charles Mickle Fellowship, in 1923; the Reeve Prize, in 1923—all of the University of Toronto; the Nobel Prize, in 1923; the John Scott Medal, by the City of Philadelphia, in 1923; the Rosenberger Gold Medal, by the University of Chicago, in 1924; the Cameron Prize, by the University of Edinburgh, in 1927; the Flavelle Medal of the Royal Society of Canada, in 1931; the Apothecaries' Medal of London, in 1934; the F. N. G. Starr Gold Medal of the Canadian Medical Association, in 1936. In 1934, Dr. Banting was made Knight Commander of the Civil Division of the Order of the British Empire. He was a licentiate of the Royal College of Physicians, a member of the Royal College of Surgeons, a fellow of the American College of Physicians, a fellow of the Royal College of Surgeons of England, a fellow of the Royal College of Surgeons of London, a fellow of the Royal College of Surgeons of Canada, a fellow of the Royal Society, a fellow of the Royal College of Physicians, an honorary fellow of the Academy of Medicine of Toronto and of the New York Academy of Medicine and an honorary member of the Norwegian Medical Society of Oslo. Dr. Banting was also made a foreign correspondent of the Académie royale de médicine de Belgique and the Società mèdico-chirurgica de Bologna, a corresponding member of the Royal Medical Society of Budapest and

an honorary corresponding member of the Royal Society of Medicine (Section of Therapeutics and Pharmacology). He was also a member of the Ontario and the Canadian Medical Association, the American Society for Pharmacology and Experimental Therapeutics, the British Physiological Society, the Association of American Physicians, the Canadian Chemical Association, the American Association for Cancer Research and the Imperial German Academy of Natural Sciences. He was an Alpha Omega Alpha.

One of the fine things that were done for him was the foundation and erection of the Banting Institute, which not only serves for teaching and much scientific work, particularly of the department of pathology of the University of Toronto, but is dedicated in the major part to medical research. This was done through the establishment of the $1,000,000 Banting Medical Research Foundation in 1923. Dr. Banting contributed $10,000 to this foundation out of his Nobel Prize Award. The cost of the Banting Institute was contributed to by the government of the Province of Ontario, the University of Toronto and the Banting Medical Research Foundation and by popular subscription. There, as head and professor of the chair of medical research, he gathered about him, after the opening of the institute in 1930, groups of men eager in the solution of great problems of medicine, men who have brought the name of the Banting Institute prominently before the world of medical science. Today Sir Frederick's papers number almost fifty. Many of them were written in collaboration with other men, such as Drs. C. H. Best, J. J. R. MacLeod, J. B. Collip, W. R. Campbell, A. A. Fletcher, E. C. Noble, S. Gains, G. H. Ettinger and G. E. Hall.

Through my brother, Dr G. E. Hall, then associate professor of medical research but now on leave of absence with the Royal Canadian Air Force, I met Dr. Banting and came to know him well. Although he was a quiet, unassuming man, he impressed me as one of a strong personality, and it was easy to understand how he was able to fire those about him with his enthusiasm and eagerness in the pursuit of research problems, and why his men loved and admired him. He was always so ready and anxious to assist and give credit to others. After the development of insulin, the energies of Dr. Banting and his associates were directed prominently toward silicosis, a definite mining problem in Canada, and toward coronary heart disease and knowledge of the adrenal cortex. All of these researches have been of importance to the world of medicine in general and have dealt with outstanding problems in pathology as well. Delving into conditions familiar to the pathologist, he made the pathologic solutions applicable in the clinical alleviation of these conditions. Above all else, however, his efforts were concentrated on the problem of cancer. His closest associates say that the results of his work on cancer and of that on silicosis will be of the greatest impor-

tance. Who can say what might have been accomplished if fate had allowed this man to carry on? With the coming of the Second World War, he turned his abilities fully to the solution of physiologic problems of grave importance in the changing phases of war, particularly those concerned with aviation and the problem of the "blackout." He again entered the Canadian Army Medical Corps, serving as a major. Early in the war, he spent considerable time in London, particularly in association with the War Department and the National Research Council. On his return to Canada, I had understood he was returning to the administrative and research functions of his chair in the university. Apparently, however, the return was just a further extension of his newer work, and on Feb. 21, 1941 he met his accidental death, answering the call of his empire.

The pilot's report of the accident is particularly tragic. Dr. Banting was not killed outright but lived for some sixteen or twenty hours, dying of the accumulating effects of what I have been told was a compound of cerebral and cranial injury, fractured ribs and punctured lungs. Much of the time he was unconscious, but in his semilucid intervals he struggled to set down or dictate to the injured pilot beside him technical and medical matters. He gave strongly the impression that he was carrying on at a post of duty. At these times his speech appeared quite coherent, but he dealt with medical problems beyond his companion's comprehension. It is not clear if he knew where he was or what had happened. Sometimes he acted as though he were a military officer or perhaps a lecturer before a clinic. From time to time, rousing himself, he would order his pilot to take down his dictation, dictating rapidly, in an apparently lucid condition, letters, memoranda and statements, all of which were merely streams of wholly unintelligible technical medical phraseology, which the injured pilot could only make a pretense of writing. Scientific and medical information of the most priceless character may well have been lost in those few hours. For it was a race against time and death by a great mind struggling to record last thoughts. Did these concern the results of the important research which had preceded his interrupted journey to England, so desperately important to his country, or plans and ideas and unrecorded projects of other research problems which had filled his mind, postponed for more peaceful days? We shall never know.

Sir Frederick's hobby was painting, and today his canvases are greatly prized. "Parergon," a booklet illustrating some of the achievements of today's physicians in the field of fine arts, presents two of his paintings, "Maligne Lake, Canada," and "Mary Lake."[1] His art works,

1. Banting, F. G.: Parergon, ed. 2, Evansville, Ind., Mead Johnson & Co., 1938.

chiefly landscapes, were characterized by sharp, incisive contrasting colors and greatly resemble the very distinctive and peculiar works of the group of contemporary Canadian artists known as the "Group of Seven."

Dr. Banting's monument will always be insulin, which has become a sure therapeutic weapon in the treatment of the once dreaded diabetes and more recently of dementia praecox. But one knows there could have been much more. As expressed in one of the letters sent me about his death, it is "another angle of our existence that we cannot understand. A man who is doing a great work that is for the betterment of humanity passes on in the prime of his life, and then we look around and see the comparison in some of the apparently useless and aimless characters that live long to clutter the earth with their selfishness." One man has sought to build and to do good; another, a mad man, seeks to destroy; and, because of that mad man, a great man has been lost to the world.

Dr. Banting is survived by his son, William, and by Lady Banting of his second marriage. The world will have to wait long to replace him.

WILLIAM E. B. HALL, M.D.

Book Reviews

Pneumonia with Special Reference to Pneumococcus Lobar Pneumonia. Roderick Heffron, M.D., Medical Associate, the Commonwealth Fund; formerly Field Director, Pneumonia Study and Service, Massachusetts Department of Public Health. Cloth. Pp. 1086. Price $4.50. New York: The Commonwealth Fund, 1939.

This book is an outcome of the Massachusetts Pneumonia Study and Service, conducted by the State Department of Public Health during the years 1931 to 1935 and financed by the Commonwealth Fund. In his foreword Dr. Frederick T. Lord says that "the success of this project in the state-wide extension of specific therapy of pneumococcus pneumonia was in large measure due to the enthusiasm and ability of Dr. Heffron, who served as Field Director." The book contains an enormous amount of well arranged, well digested and fluently presented information about pneumonia. It is the third book dealing with results of the Massachusetts study of pneumonia. In its preparation "much of the literature relating to pneumonia was reviewed and abstracted in detail," as illustrated by the 1,471 titles in the bibliography. The elaborate analytic index will be of great help in the use of the book as a source of information. No better general characterization of the book can be offered than that in Dr. Lord's foreword: "The book is destined to fill an important place in the literature on pneumonia. It brings together in one place a comprehensive review of investigations dealing with the inciting agent, lesions produced, clinical aspects, factors influencing recovery, prevention and treatment. It is especially valuable as a source of information concerning the results of specific therapy in large series of collected cases, including those observed during the Massachusetts Pneumonia Study and Service and the Continuing Program. The need for further investigation of special problems is pointed out. . . . The book contains helpful suggestions concerning the further extension of antipneumococcic serum as a community project."

Medical Microbiology. Kenneth L. Burdon, Ph.B., Sc.M., Ph.D., Assistant Professor of Immunology and Bacteriology, Louisiana State University School of Medicine, New Orleans. Cloth. Pp. 763, with 120 illustrations. Price $4.50. New York: The Macmillan Company, 1939.

This book is divided into four parts. Part I is concerned with the fundamentals of microbiology and covers 238 pages. Part II deals with the laboratory study of micro-organisms and occupies 62 pages. Part III is concerned with infection and resistance and comprises 132 pages. Part IV deals with the microbiology of important diseases, to which 220 pages are given. The rest of the book is made up of appendixes and the index.

The arrangement of the subject matter departs from the conventional in several respects. In the opening chapters, the reader is introduced to all types of micro-organisms, to protozoa, spirochetes, rickettsias, fungi, moldlike higher bacteria and filtrable viruses as well as to the ordinary true bacteria. Immunologic and epidemiologic considerations precede discussions of particular disease processes. The traditional method of arrangement according to a systematic classification of the bacteria is discarded, and the individual diseases are introduced under the heading of the region of the body principally or primarily affected.

No bibliography accompanies the text, but references to books and current periodicals are given in an appendix.

The direct dogmatic manner in which this text is written leaves little to the imagination of the reader and certainly belies the fact that the subject matter of bacteriology is highly controversial. However, for the beginning student of medical bacteriology this work has much in its favor.

Books Received

DISEASES TRANSMITTED FROM ANIMALS TO MAN. Thomas G. Hull, Ph.D., director of the American Medical Association Scientific Exhibit. Second edition, XIII. Pp. 403, with 45 figures. Price $5.50. Springfield, Ill.: Charles C. Thomas, Publisher, 1941.

NATIONAL RESEARCH COUNCIL: ORGANIZATION AND MEMBERS, 1940-1941. Washington, D. C.: National Research Council, December 1940.

STUDIES ON TUBERCULOSIS: THE SPREAD OF TUBERCULOSIS IN NEGRO FAMILIES OF JAMAICA, B.W.I. E. Joyce Saward, Persis Putnam and Eugene L. Opie. THE FATE OF NEGRO PERSONS OF A TROPICAL COUNTRY, JAMAICA, B.W.I., AFTER CONTACT WITH TUBERCULOSIS. Eugene L. Opie, Persis Putnam and E. Joyce Saward. A SURVEY OF TUBERCULOUS INFECTION IN A RURAL AREA OF EAST ALABAMA. A. H. Graham, P. W. Auston and Persis Putnam. THE FATE OF PERSONS EXPOSED TO TUBERCULOSIS IN WHITE AND NEGRO FAMILIES IN A RURAL AREA OF EAST ALABAMA. A. H. Graham, P. W. Auston and Persis Putnam. The American Journal of Hygiene Monographic Series, no. 16, February 1941. Supported by the De Lamar Fund of the Johns Hopkins University. The publication of this monograph (no. 16) was financed by the Rockefeller Foundation, New York. Pp. 198. Baltimore: The Johns Hopkins Press, 1941.

THE AVITAMINOSES: THE CHEMICAL, CLINICAL AND PATHOLOGICAL ASPECTS OF THE VITAMIN DEFICIENCY DISEASES. Walter H. Eddy, Ph.D., professor of physiological chemistry, Columbia University Teachers College; director, Good Housekeeping Magazine, Bureau of Foods and Sanitation. Gilbert Dalldorf, M.D., pathologist to the Grasslands and the Northern Westchester Hospital, Westchester County, New York. Second edition. Pp. 519, with 41 plates. Baltimore: Williams & Wilkins Company, 1941.

SCIENCE AND SEIZURES: NEW LIGHT ON EPILEPSY AND MIGRAINE. William Gordon Lennox, M.D., Sc.D., Hon., assistant professor of neurology, Harvard Medical School; visiting neurologist, Boston City Hospital; president, International League Against Epilepsy; vice president, Laymen's League Against Epilepsy; secretary, Harvard Epilepsy Commission. Pp. 258, with 10 illustrations. Price, $2. New York: Harper & Brothers, 1941.